August 16, 2018

Ms. Christina Motilall
Risk Assessment Division
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington, DC 20460

Submitted via the Federal eRulemaking Portal: https://www.regulations.gov/

Re: EPA-HQ-OPPT-2016-0723, EPA-HQ-OPPT-2016-0725, EPA-HQ-OPPT-0732, EPA-HQ-OPPT-2016-0733, EPA-HQ-OPPT-2016-0735, EPA-HQ-OPPT-2016-0736, EPA-HQ-OPPT-0737, EPA-HQ- OPPT-2016-0741, EPA-HQ-OPPT-2016-0742, and EPA-HQ-OPPT-0743

Dear Ms. Motilall,

The American Chemistry Council (ACC) is pleased to submit the attached comments on EPA's problem formulations for risk evaluations to be conducted on the initial 10 chemicals under TSCA.

These comments align with our comments on the proposed Application of Systematic Review in TSCA Risk Evaluations, which have been separately filed and are also attached here as Attachment B. These comments should be considered together.

Please let me know if you have any questions regarding these comments. I can be reached at 202-249-6440 or by email at Suzanne_Hartigan@americanchemistry.com.

Sincerely,

Suzanne Hartigan

Suzanne Hartigan, Ph.D.
Senior Director
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AMERICAN CHEMISTRY COUNCIL COMMENTS ON THE PROBLEM FORMULATIONS FOR THE U.S. ENVIRONMENTAL PROTECTION AGENCY'S INITIAL 10 CHEMICALS IDENTIFIED FOR RISK EVALUATION

August 2018

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Executive Summary

The American Chemistry Council (ACC)[1] appreciates the opportunity to provide comments on the problem formulations for the initial 10 chemicals to undergo risk evaluation under the amended Toxic Substances Control Act (TSCA). ACC supports EPA's practical and thoughtful approach to reviewing the circumstances of manufacturing, processing, distribution, use, and disposal of these chemicals to inform the development of the scoping process. The problem formulations show the Agency's commitment to identifying and reviewing those conditions of use that represent the greatest potential for risk and document its efforts to gather the best available information to use in the risk evaluations.

ACC remains committed to the efficient and effective implementation of the TSCA amendments and makes several recommendations to the Agency for its consideration in these and future scoping documents for TSCA risk evaluations:

- **Systematic Review:** EPA's development of a structured process to identify, evaluate, and integrate evidence from both the hazard and exposure assessments developed during the TSCA risk evaluations is appropriate and will provide increased transparency into the TSCA risk evaluation process.
- **Conditions of Use**: EPA should develop a framework for its scoping approach that articulates its process for deciding when conditions of use will be in or out of scope of the risk evaluation. This would help streamline EPA's future efforts, increase transparency, and help industry anticipate EPA's information needs in the risk evaluations.
- **Coordination with Other Appropriate Federal Executive Departments or Agencies:** EPA should describe its coordination with other federal agencies—OSHA in particular—to clarify how it will undertake its TSCA Section 9(d) consultation obligations. This coordination is essential to avoid duplicative and unnecessary regulation.
- **Tiered Approaches to Assessment**: EPA should apply tiered approaches throughout the risk evaluation process to enable EPA to meet TSCA's deadlines, adhere to TSCA's science standards, and enable both EPA and the regulated community to apply limited resources efficiently.
- **Road Map and Guidance on Tiered Exposure Assessments**: A road map showing EPA's approach to tiered exposure assessments and related guidance would be useful. Moreover, EPA should be more transparent about the specific exposure models, occupational exposure limits, and margins of exposure it intends to rely upon in its risk

[1] The American Chemistry Council (ACC) represents the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier and safer. ACC is committed to improved environmental, health and safety performance through Responsible Care®; common sense advocacy designed to address major public policy issues; and health and environmental research and product testing. The business of chemistry is a $768 billion enterprise and a key element of the nation's economy. It is among the largest exporters in the nation, accounting for fourteen percent of all U.S. goods exports. Chemistry companies are among the largest investors in research and development. Safety and security have always been primary concerns of ACC members, and they have intensified their efforts, working closely with government agencies to improve security and to defend against any threat to the nation's critical infrastructure.

evaluations. To ensure consistency across the Agency's exposure assessments, EPA should rely upon standard exposure scenarios.

- **Occupational Exposure**: EPA should offer more detail on the data and information it plans to consider in its occupational exposure assessments. EPA should provide broader guidance on how it will evaluate occupational exposures under TSCA, including consideration of the most up-to-date information.
- **Ecological Exposures:** EPA should explain its tiered approach to ecological exposures and consider further standardization of emission scenarios and environmental release categories, including updating Generic Scenarios used in conjunction with ChemSTEER.
- **Consumer Exposures**: EPA must ensure that its models are publicly available, accessible, and can be readily used by knowledgeable professionals. Further, EPA should provide greater clarity about how it will assess consumer exposure to chemicals in products.
- **Hazard Assessment**: EPA should use integrated hazard assessments for regulatory decision making, and should work to identify, develop, and integrate non-animal methods. EPA should consider mode of action (MOA) in problem formulations and, where appropriate and supported by the data, ACC recommends EPA adopt and apply the World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) MOA framework in risk assessments.
- **Ecological Hazard Assessment**: EPA should identify the higher-tier approaches it may use for determining a hazard threshold, especially for data rich chemicals.

Each of these recommendations is discussed in more detail below.

I. General Considerations

A. EPA Must Use a Structured Process to Identify, Evaluate, and Integrate Hazard and Exposure Information Developed During the TSCA Risk Evaluations.

Section 26 of TSCA mandates that EPA make science-based decisions under Sections 4, 5, and 6 of TSCA in a manner consistent with the best available science and the weight of the scientific evidence.[2] EPA's development of a structured process to identify, evaluate, and integrate evidence from both the hazard and exposure assessments developed during the TSCA risk evaluations is appropriate and will provide increased transparency into the TSCA risk evaluation process. Systematic review approaches applied in many fields provide frameworks to identify, select, evaluate, and integrate scientific evidence to support a conclusion.[3] ACC's comments on EPA's proposed approach, "Application of Systematic Review in TSCA Risk Evaluations,"[4] (Systematic Review) have been filed in that docket. Those comments are also attached here and

[2] 15 U.S.C. §§ 2625(h) and (i).

[3] Wikoff, D.S. and Miller, G.W. 2018. Systematic reviews in toxicology (Editorial). Toxicological Sciences, 163(2): 335-37.

[4] US EPA, Application of Systematic Review in TSCA Risk Evaluations, Office of Chemical Safety and Pollution Prevention, Office of Pollution Prevention and Toxics, EPA Document # 740-P1-8001. Available at https://www.epa.gov/sites/production/files/2018-06/documents/final_application_of_sr_in_tsca_05-31-18.pdf

incorporated by reference to these comments on the problem formulations.[5] In general, EPA should make the results of its systematic review process available as part of the docket for each risk evaluation, including its selection of key studies and study quality evaluations.

B. Scoping Decisions Should Consider Best Available Information and Greatest Potential for Risk.

EPA has identified those conditions of use that will be within the scope of the risk evaluations, as well as those that will be excluded. The risk evaluation rule makes clear that EPA should focus on those conditions of use that raise the greatest potential for risk.[6] ACC generally supports the approach taken to addressing conditions of use within each of the 10 problem formulations. This approach allows EPA to be efficient, while still addressing the highest priority conditions of use that pose the greatest potential risk.

The problem formulation documents present a thoughtful approach to identifying current uses that are appropriate for inclusion within the scope of the risk evaluation. We also appreciate EPA's efforts to explain why the conditions of use that are not within scope will be excluded. ACC encourages continued stakeholder engagement with manufacturers and users of these chemicals throughout the risk evaluation process to ensure the best available information is used.

As EPA gains more experience conducting TSCA risk evaluations for high priority chemicals, it would be useful if the Agency would develop a framework that articulates its process for deciding when conditions of use are in or out of scope. This would help EPA streamline future efforts, provide greater public understanding of EPA's decisions, increase transparency and reproducibility, and enable industry to identify the types of information that may be most helpful for manufacturers, processors, and downstream users to develop and/or share with EPA. Developing a framework would also help industry anticipate which conditions of use will be the likely focus in future assessments so that they can direct resources efficiently to develop and/or gather information relevant to EPA's potential risk evaluations and facilitate proactive data collection efforts.

C. EPA Should Coordinate Early With Other Appropriate Federal Executive Departments or Agencies.

Section 9(d) of TSCA imposes a general requirement on EPA to consult and coordinate with other federal agencies for purposes of "achieving the maximum enforcement" of TSCA while imposing the "least burdens of duplicative requirements on those [subject to TSCA]." This Section 9(d) coordination requirement has existed since TSCA was originally enacted and was unchanged by the 2016 amendments. Section 9(d) is a general policy directive that applies to EPA for all TSCA implementation activities. The risk evaluation rule also contains a general consultation provision that codifies the statutory requirement for interagency collaboration during the risk evaluation process.[7]

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[5] See Attachment A, American Chemistry Council Comments on the Application of Systematic Review in TSCA Risk Evaluations, Docket ID EPA-HQ-OPPT-2018-0210.

[6] EPA Procedures for Chemical Risk Evaluation Under the Amended Toxic Substances Control Act, 82 Fed. Reg. 33726 (July 20, 2017).

[7] 40 C.F.R. § 702.39.

The principle driving this coordination requirement is that EPA should avoid imposing unnecessary or duplicative burdens on regulated entities and avoid regulatory actions best taken by another agency or under other EPA authority. This necessarily includes all manner of Agency interaction with regulated entities, including submission of information, docket management, responses to comments, and other engagement with multiple regulatory bodies. Where non-TSCA regulatory schemes are sufficiently effective at addressing risk, EPA may properly exclude covered conditions of use from the scope of the risk evaluation.

Regarding occupational exposures, EPA should consult early with OSHA in the risk evaluation process—certainly at the earliest stages of the risk evaluation and well before the scope is released.[8] This consultation should continue throughout the risk evaluation. None of the 10 problem formulations make clear what consultation may have occurred, or when it occurred. Although the problem formulations do identify available occupational exposure levels (OELs), i.e., PELs, TLVs, and IDLH values, additional information should be provided regarding the factors EPA will take into consideration when evaluating OELs. For example, consideration should be given to whether the OEL includes current toxicological and epidemiological data to support the development of the threshold limit value.[9] EPA also presents summarized personal monitoring air samples obtained from OSHA inspections, but it is not clear how these data were obtained from OSHA and under what circumstances the data were gathered. EPA should give preference to direct data obtained for uses being evaluated with consideration given to how the data were gathered (i.e., workplace exposure monitoring data are gathered on a more routine basis while OSHA monitoring is conducted typically in compliance with the OSHA Technical Manual for 8 hours and the sample will generally involve the scenario or tasks in which the highest exposure is expected).

For purposes of 9(d) compliance, it would be helpful if subsequent risk evaluation scopes offer more detail regarding EPA's coordination with other agencies, including information such as consultation plans, data shared, etc. We encourage EPA to include such a coordination plan in future scopes and to include these plans in the draft risk evaluations, including notations where consultation has occurred. It would be helpful for EPA to describe the decision criteria/framework by which it will evaluate whether to include occupational exposures in the scope of a risk evaluation. This description was not included in the 10 problem formulation documents.

D. EPA Must Use Tiered, Iterative Approaches to the Risk Evaluation Process.

EPA should apply a tiered approach throughout the risk evaluation process–from screening/prioritizing chemicals to conducting risk evaluations–under amended TSCA. This is essential to enable EPA to meet TSCA's statutory deadlines for completing risk evaluations,

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[8] To the extent that other regulatory authorities are relevant to a particular risk evaluation, the same principle of early consultation should apply. We focus on OSHA here due to the consideration of occupational exposure within the scope of all 10 problem formulations, although only nine will further analyze occupational exposure in the risk evaluation.

[9] Any OELs imposed by EPA as regulatory requirements must be ones that have been subject to notice and comment and suitable for regulatory purposes.

adhere to TSCA's robust scientific standards, and enable both EPA and the regulated community to apply limited resources efficiently.

When a screening-level assessment is insufficient to conclude a lack of risk to exposed populations, EPA should take steps to refine the risk evaluation allowing more accurate quantification of potential risks. The scoping/problem formulation documents indicate where the EPA feels it has sufficient information and where additional information and use of higher-tier tools is warranted. In situations where EPA may need to perform higher-tier assessments for the risk evaluation, more information is needed on the types of data and techniques that EPA will utilize. For example, EPA should indicate how probabilistic risk assessment (PRA), uncertainty analyses, and the use of statistical tools such as Bayesian statistics would be used at a higher tier within the overall problem formulation framework. A tiered, iterative approach is critical to the production of high quality risk evaluations based on the best available information.

II. Exposure Assessment Considerations

A. EPA Should Clearly Define How it Plans to Apply Tiered Exposure Assessments in TSCA Risk Evaluations, Beginning with the Initial 10 Chemicals.

Consistent with our recommendation above in Section I.D., EPA should apply tiered exposure assessments in its risk evaluations of high priority TSCA chemicals and clearly define its planned tiered exposure approach in EPA's problem formulation documents.

1. The Value of Tiered Exposure Assessments Is Widely Recognized.

The value of tiered exposure assessment is well-established. In its 1992 guidelines on exposure assessment,[10] EPA discusses the value of tiered exposure assessments from screening-level assessments to more complex assessments. This perspective was reiterated in EPA's 2016 peer review draft update of the 1992 guidelines.[11] The 2016 draft update included specific discussion of considerations in tiered assessments, as well as the notion of "fit for purpose" assessments, stating "[t]he type and purpose of an exposure assessment determine the data and information requirements."[12] The EPA Office of Research and Development (ORD) ExpoBox tool box for exposure assessors identifies exposure assessments tools by tier and type, both screening-level and refined, for planning, scoping, and problem formulation.[13]

The purpose of tiered exposure approaches is well understood: to identify uses of chemicals that, under very conservative (e.g., maximum) exposure assessment assumptions, are not likely to pose a health risk.[14] Depending on the conditions of use, the exposure assessment information

[10] US EPA, Guidelines for Exposure Assessment, Risk Assessment Forum, Washington, DC, EPA/600/Z-92/001, 1992.
[11] US EPA, Guidelines for Human Exposure Assessment, Risk Assessment Forum, Peer Review Draft, January 7, 2016.
[12] *Ibid*, at 2.
[13] https://www.epa.gov/expobox/exposure-assessment-tools-tiers-and-types-screening-level-and-refined.
[14] For example, see Patlewicz, G., et al. 2018. Utilizing Threshold of Toxicological Concern (TTC) with high throughput exposure predictions (HTE) as a risk-based prioritization approach for thousands of chemicals.

can be used either to identify a chemical as a low priority or to be factored into the overall risk evaluation. Exposures that initially exceed hazard benchmarks in Tier-1 exposure assessments would require more refined, higher-tiered approaches to exposure assessments. This would include the application of more realistic parameters related to the likely duration, intensity, frequency, and number of exposures and more realistic exposure scenarios to more accurately quantify actual risks of the chemical.

The importance of EPA using a tiered approach to exposure assessment in its TSCA risk evaluations cannot be overstated. A tiered approach allows for both a more rapid, yet systematic, approach for assessing conditions of use in a first-tier screen, so that resources are used effectively when a refined exposure assessment is necessary for those conditions of use that do not "pass" a first-tier screen. A well-defined, tiered exposure approach can lead to greater efficiencies in chemical risk evaluations under TSCA. Congress clearly valued such efficiency highly as evidenced by the aggressive deadlines it set for EPA to conduct TSCA risk evaluations. Congress also directed the Agency to consider the likely duration, intensity, frequency, and number of exposures under the conditions of use.[15]

The value of tiered exposure approaches in risk evaluations is even broader than exposure assessment. This was discussed in the Health and Environmental Sciences Institute's (HESI) Coordinated Risk Assessment in the 21st Century (Risk21) project. A review article published in 2014 discussing Risk21's principles and framework for decision-making in human health risk assessment emphasizes that problem formulation for risk assessment should not be a hazard-driven process, but instead should start with exposure, focusing on exposure scenarios of greatest concern integrated with hazard information to support risk-based decision making. [16] The article suggests this approach would result in an early estimate of potential human exposure in relevant populations, including susceptible populations, which would characterize the degree of specific toxicological data needs.[17] The Risk21 framework also addresses two other principles: (1) additional data should be acquired "only if necessary and when they add value"[18] and (2) flexibility, "such that a higher tier hazard assessment approach can be coupled with a lower tier exposure approach, and vice versa."[19,20] Considerable progress has been made over the last several years in developing screening-level exposure prediction models for chemicals in commerce.[21] These approaches can be of particular utility in conducting Tier-1 assessments for many chemicals.

Computational Toxicology, 7: 58-67. Available at https://www.sciencedirect.com/science/article/pii/S2468111318300689

[15] 15 U.S.C. § 2605(b)(4)(F)(iv).

[16] Pastoor, T.P., et al. 2014. A 21st century roadmap for human health risk assessment. Critical Reviews in Toxicology, 44: 1-5. Available at https://www.tandfonline.com/doi/full/10.3109/10408444.2014.931923.

[17] *Ibid,* at 3.

[18] *Ibid.*

[19] *Ibid.*

[20] National Research Council. 2009. Science and Decisions: Advancing Risk Assessment. Washington, DC: The National Academies Press. Available at https://doi.org/10.17226/12209.

[21] For examples, see https://pubs.acs.org/doi/pdf/10.1021/acs.est.5b00498; https://pubs.acs.org/doi/abs/10.1021/es400482g; https://www.ncbi.nlm.nih.gov/pubmed/25222184

In the context of TSCA's risk evaluations, tiered-assessment concepts equip EPA with the tools it needs to meet TSCA's aggressive deadlines for completing risk evaluations of high priority chemicals. Tiered assessments also enable EPA to apply limited resources in an efficient manner. Using a clear, science-based tiered-assessment approach, EPA and the regulated community can perform exposure assessments in TSCA risk evaluations, enabling efficient decision-making.

2. A Road Map on Tiered Exposure Assessments Is Needed.

The draft problem formulation documents of the initial 10 chemicals mention the Agency's plans to use tiered exposure assessments in its risk evaluations of these chemicals, but the documents lack specifics. A clear "road map" showing EPA's approach to tiered exposure assessments is needed in EPA's scoping documents. Such a road map—or decision tree—would provide structure to EPA's approach to exposure assessments under TSCA. This structure would also be useful to explain how EPA will integrate the results of its tiered exposure assessments with the results from its tiered-hazard assessments in TSCA risk evaluations.

A road map would signal to the regulated community the type of reasonably available exposure information EPA plans to rely upon, what additional exposure information might be needed, and what actions manufacturers could take early in the risk evaluation process to provide EPA the needed exposure information. EPA should delineate what kinds of data and information it could accept to refine lower-tier exposure assessments.

Specifically, with respect to potential human exposures in the problem formulation documents, EPA should identify:

- The screening-level exposure information/models EPA will use to address human exposure in Tier-1 exposure assessments;
- The approach to hazard characterization and threshold EPA will use to ascertain the need for a higher-tier exposure assessment;
- How EPA will communicate Tier-1 exposure screening-level results;
- The higher-tiered information and models EPA will use to address human exposures, suggested by the results of the screening-level information/models;
- How EPA might use tiered exposure evaluations for specific exposure scenarios (e.g., occupational, consumer, residential, etc.)
- What kind of data and information EPA would accept (i.e. from stakeholders) to refine a Tier-1 screening exposure assessment.

3. EPA Guidance on Tiered Exposure Assessment Is Needed.

TSCA Section 26(l) requires EPA to develop "policies, procedures and guidance that the Administrator determines are necessary to carry out the amendments" of amended TSCA. EPA indicates its intent to use tiered approaches in TSCA risk evaluations, but guidance is needed. EPA should develop new, more specific guidance on its plans to use tiered approaches to exposure assessment in TSCA risk evaluations.

In doing so, EPA must move beyond mere "concepts" and reference lists to specific information, models, and tools. Specific and transparent guidance is needed to understand how the Agency will conduct its exposure assessments so that manufacturers can provide the most relevant information early on in the process to the Agency and so that stakeholders understand the process. As stated earlier, EPA should indicate how PRA, uncertainty analyses, and the use of statistical tools would be integrated as a higher tier assessment. Such guidance will also allow stakeholders to provide additional information to refine initial lower tier exposure estimates.

Further program-specific guidance is also needed for those manufacturers that plan to conduct risk evaluations for EPA's consideration and must conform to EPA's approach to risk evaluations should they do so. Guidance on tiered approaches will help streamline the risk evaluation process under TSCA and enable EPA to meet TSCA's new mandates.

Canada's Chemical Management Plan (CMP),[22] Australia's Inventory of Chemical Substances,[23] and the EU's Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) program[24] employ tiered approaches in their exposure assessment approaches for chemicals. EPA should review those approaches to ascertain their usefulness in new EPA guidance on tiered exposure assessments in TSCA risk evaluations.

B. Occupational Exposures

According to EPA's problem formulations, EPA plans to further analyze occupational exposures in nine of the 10 chemicals risk evaluations. EPA must be more transparent about its coordination with OSHA regarding its plans to address occupational exposure issues in TSCA Section 6 risk evaluations. The methods, models, and databases that the Agency uses to conduct its occupational exposure assessments must be adequate to satisfy TSCA's Section 26 standards for best available science and weight of the scientific evidence. EPA should be more transparent about the OSHA and NIOSH databases that EPA plans to rely upon in these risk evaluations. Greater transparency will provide manufacturers notice about the type of information EPA may not have, but may need, to conduct a realistic occupational exposure assessment.

1. EPA Should Discuss in More Detail the Databases It Plans to Rely Upon in Its Occupational Exposure Assessments in Its Risk Evaluations.

In eight of the problem formulation documents, EPA has identified OSHA's Chemical Exposure Health Data (CEHD) and NIOSH's Health Hazard Evaluation (HHE) program data as two major sources of occupational monitoring data that it will rely upon in the risk evaluations. However, EPA does not discuss what information in these databases it plans to rely upon; how representative the data are; what criteria EPA will use in deciding which data are or are not applicable for its exposure assessments; or how it plans to assess those data in the context of

[22] https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/chemicals-management-plan-risk-assessment-toolbox.html
[23] https://www.nicnas.gov.au/__data/assets/word_doc/0018/35127/Inventory-Multi-tiered-Assessment-and-Prioritisation-Framework-Review-Document.docx
[24] https://echa.europa.eu/documents/10162/13632/information_requirements_part_d_en.pdf/70da6d4b-5acf-40d9-8b75-1e1c311378df

current OSHA regulations and industrial hygiene practices. EPA must provide greater detail about its use of the information in these OSHA and NIOSH databases to enable stakeholders to comment upon the data quality for the purposes for which EPA plans to rely upon the data, and to provide the Agency higher quality data where it exists.

For instance, it is our understanding that the OSHA CEHD information does not include a description of the activities associated with the specific exposure measurements. Without this information, how will EPA be able to apply these results to the conditions of use identified for a chemical? Absent sufficient knowledge of activities associated with occupational exposure measurements, EPA might very well improperly assign exposure values to a certain condition of use/application. This could result in inappropriate conclusions about risk under specific conditions of use or risk management recommendations for protection of workers. It appears that this database reports non-detects (ND), but it does not specify the limit of detection (LOD). Without an understanding of the accuracy of the data, how will EPA use this data to inform estimates of exposure?

In occupational settings, potentially hazardous exposures are eliminated or minimized by the use of training, industrial hygiene programs, engineering controls, closed systems, personal protective equipment (PPE), labeling, medical surveillance, etc. Over the past several decades, these engineering and industrial hygiene practices have continually improved. For example, as part of ACC's Responsible Care® Program, ACC member companies must implement ACC's Process Safety Code, which aims to supplement existing process safety requirements contained within the Responsible Care Management System® and RC14001® technical specifications. The Process Safety Code is intended to complement regulatory standards that, by necessity, focus on process safety at an individual facility.[25]

Another concern with the OSHA CEHD database is that much of the data were developed during inspections of facilities suspected of having high employee exposures. This suggests these data are not representative of occupational exposures from facilities that are in compliance with OSHA standards. EPA should address this fact in its quality review of the data/information underpinning its risk evaluations.

ACC understands that some ACC members have provided EPA with occupational monitoring information for use by the Agency in problem formulations for some of the initial 10 chemicals, but this information was apparently not reflected in the problem formulations issued on June 11, 2018. EPA should be clear in the draft risk evaluations how such submitted occupational monitoring information was used to prepare the problem formulations and considered in the risk evaluation.

[25] The ACC Process Safety Code is more universal, as it addresses issues across a division or corporation and includes a company commitment to set process safety expectations, define accountability for process safety performance, and allocate appropriate resources to achieve performance expectations. ACC members are required to demonstrate conformance to the Responsible Care management system as an obligation of membership. Conformance, including implementation of the ACC Process Safety Code, is demonstrated through ACC's mandatory third-party certification audit process.

2. EPA Should Provide Broader Guidance on How It Will Address Occupational Exposure Under TSCA.

EPA indicates it plans to further analyze occupational exposures in the draft risk evaluations in nine of the 10 problem formulations. EPA has conducted very few worker exposure assessments on existing TSCA chemicals in the past and its *Exposure Factors Handbook* does not address occupational exposures.[26]

EPA has occupational exposure tools that are designed for specific purposes. For example, ChemSTEER was developed as a conservative screening tool used to estimate workplace exposures and environmental releases for new chemicals that are manufactured and used in industrial/commercial settings. However, broad guidance is not currently available for evaluating occupational exposures under TSCA, in particular with respect to the evaluation of existing chemicals. EPA should develop new guidance for evaluating occupational exposures under TSCA.

To develop this guidance, EPA should certainly consider its own information, models, and tools on occupational exposure. EPA should also update some of its older tools and methods to evaluate worker exposure. EPA should update its 1997 Generic Scenarios for industry-specific workplace release and exposure estimation to make certain they reflect current industry practice. Many industrial practices in use today go beyond the legal regulatory requirements of OSHA. EPA should consider current industrial hygiene practices as part of the conditions of use of manufacturing. Additional Generic Scenarios may need to be developed to cover conditions of use for which Generic Scenarios do not currently exist.

It is also critical that EPA consider other information and tools available from OSHA, from the American Industrial Hygiene Association (AIHA), and from other jurisdictions to develop new occupational exposure guidance for TSCA purposes. EPA should consider the applicability of new models being used in Canada and the EU in their chemical regulatory programs.[27] In considering information and tools from OSHA, AIHA, and other jurisdictions, EPA should also consider the adequacy and appropriateness of use of those tools in the TSCA context.[28]

With respect to dermal exposures, the problem formulation documents identify several models for application to four of the 10 chemicals.[29] EPA's existing dermal exposure assessment guidance is primarily geared toward neat compounds in soil or water, and it is not clear whether this guidance is sufficient to evaluate chemicals encountered in industrial-use scenarios. For inhalation exposures, EPA has identified several models it plans to use in nine of the problem formulations.[30] EPA guidance on potential inhalation exposures in occupational conditions of use under TSCA would be helpful.

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[26] US EPA. Exposure Factors Handbook 2011 Edition (Final Report). Washington, DC, EPA/600/R-09/052F, 2011.
[27] For example, see ECHA's Advanced REACH Tool (ART) at https://www.advancedreachtool.com/.
[28] Any tools and information EPA would seek to adopt in its guidance should be available for notice and comment to ensure consideration public input.
[29] These include the ChemSTEER penetration model, ChemSTEER 2-Hand Dermal contact in Liquid; ChemSTEER 2 Hand Dermal Immersion in Liquid; PBPK model (Poet et al) and ChemSTEER
[30] These include the Near-Field/Far Field model (Keil); AEROMOD; EFAST; ChemSTEER; PBPK model (Poet et al); Two-Zone model; and Two-zone Near Field/Far Field (Jayjock et al.).

Guidance on occupational exposure assessment under TSCA should address how the Agency will consider standard industrial hygiene practices as well as how that information will be incorporated into its exposure assessments and how ultimately that information will be integrated into the risk evaluation. EPA should address and identify the specific information the Agency will need to accomplish these steps; the level of detail needed to enable the Agency to reach a determination about the adequacy of design measures such as: closed systems; the use of engineering controls and labeling requirements (e.g., the use of gloves or other PPE); and other operating procedures and management practices currently in use to eliminate or adequately minimize exposures in occupational settings. EPA should describe how these considerations are incorporated into a tiered occupational exposure assessment.

EPA may need to gather information from industry regarding current occupational exposure protection practices. Industry may be able to facilitate access to that information. Manufacturers and organizations like AIHA may be able to help the Agency gather information about exposure data in occupational settings and industrial hygiene practices in various workplace situations. Ultimately, through such efforts, an EPA exposure factors handbook for occupational exposures could potentially be developed to address TSCA risk evaluation needs. Consistent with application of a tiered approach to assessing exposure, EPA should articulate what kind of data will be acceptable to refine an initial lower tier occupational exposure assessment. For example, if a screening level estimate from ChemSTEER needs to be refined, a road map (as described above) would be a key element of guidance to develop the necessary information to conduct a higher tier assessment.

3. Greater Transparency Is Needed Regarding Exposure Models, Margins of Exposure and Occupational Exposure Limits.

EPA should be more transparent about specific exposure models, margins of exposure and occupational exposure limits that it intends to utilize during the risk evaluation process. This will allow stakeholders to provide the Agency the exposure information it needs and can lead to better understanding as to how EPA will make risk determinations.

As noted above in Sections I.D. and II.A.1, ACC agrees with EPA's support for using tiered approaches generally, and in exposure modeling in particular. Under a tiered, iterative approach, screening-level tools, which are "protective by design," may be used initially. For substances that appear to present potential risks following a screening-level assessment, EPA should then proceed to use higher-tier tools. By beginning with screening-level assessments—which use more conservative assumptions and information than higher tier models—the Agency can optimize resource allocation by identifying exposure routes that present less risk early in the assessment process. When a Tier-1 screening assessment indicates low risk for a particular condition of use, the Agency should have a high degree of confidence that the potential risks are lower or perhaps nonexistent.

It is critical that EPA establish clear and consistent guidance that defines when Tier-1 model results will trigger more detailed and refined subsequent assessments. The more rigorous models require more input data and more Agency resources, but will result in more realistic exposure

assumptions. In the problem formulation documents, EPA frequently cites regulatory and non-regulatory occupational exposure limits,[31] but it neither clarifies how it would apply these limits during an exposure assessment, nor specifies a process that will be followed should the Tier-1 model results exceed these limits or margins of exposure. In the event that EPA uses threshold triggers for Tier-2 models within EPA's risk assessment process, the Agency must provide guidance regarding how it selects these values and provide stakeholders an opportunity to comment.

Similarly, EPA should specify which exposure models—for all routes and populations—it intends to use during the risk evaluation process. In the problem formulations, EPA mentions several different models, but it does not provide rigorous guidance as to which tools will be used under which circumstances. Similarly, EPA does not identify specifically what it considers to be "higher tier models." Exposure models vary in terms of the purposes for which they are used, their input requirements, and assumptions. By providing a rationale for its model selection, the Agency will afford stakeholders an opportunity to provide appropriate data and contribute relevant information to EPA during its risk evaluations. EPA also should be clear about the use of modeled vs. measured data in evaluating exposure. For example, if measured data are rejected in favor of modeled estimates, the rationale for such a decision needs to be clear.

4. EPA Should Rely on Standard Exposure Scenarios to Improve Consistency Across the Agency's Exposure Assessments.

EPA participates in the OECD's Working Party on Exposure Assessment (WPEA). In that capacity, EPA has been a global leader helping harmonize chemical use categories and developing standard exposure/emission scenario documents (ESDs) for occupational exposure assessments for chemical regulations. ACC expects that EPA will use these standard exposure scenarios in its occupational exposure assessments, but that is not clear from the problem formulation documents. EPA should clarify this point in its draft risk evaluations of these 10 chemicals and in any new guidance the Agency develops on exposure assessments under TSCA.

In addition, EPA should develop additional standard exposure scenarios for both worker and consumer exposures under TSCA. Standard exposure scenarios would assure greater consistency in EPA exposure assessments; improve exposure model parameters; and help industry understand what specific information EPA needs in exposure assessments for TSCA risk evaluations. In short, standard exposure scenarios would improve efficiencies when conducting TSCA risk evaluations, which are critical given TSCA's statutory deadlines.

EPA may want to consider stakeholder workshops to discuss ways in which standard exposure scenarios might be developed in the US. If so, EPA should also ensure that standard scenarios developed under REACH be discussed and considered at such workshops since many of these may be useful in TSCA as well.

C. EPA Should Explain What Additional Ecological Exposure Assessment Tools Are Available.

[31] Common examples include, OSHA Permissible Exposure Limits (PELs), NIOSH Recommended Exposure Limits (RELs), or ACGIH Threshold Limit Values (TLVs).

The screening-level approaches described in the problem formulation documents are appropriate for this step (i.e., E-FAST), but EPA should identify acceptable tools/methods for higher-tier refinement when necessary. Screening-level exposure analysis may be suitable in cases where estimates do not exceed the Concentration of Concern (COC). EPA should explain how it would use higher-tier information, if provided.

EPA has indicated that environmental exposure data may be available for some of these 10 chemicals in the EPA Discharge Monitoring Report tool, EPA's STOrage and RETreival (STORET) system, USGS National Water Quality Assessment (NAWQA) program, and other sources. Some of these data sources may not be current and therefore may not represent the best available information. EPA should clarify exactly how it would use such data to establish a national, regional, or local environmental exposure estimate.

EPA should also clarify how it will quantify and assess (or exclude) naturally-occurring sources of chemicals for assessment during exposure estimation.

D. Consumer Exposures

1. All EPA Models Used in Its Assessments Must be Publicly Available and Accessible.

EPA's Consumer Exposure Model (CEM) is mentioned as the preferred tool for estimating consumer exposures in several of the first 10 chemicals' risk evaluations. This model is publicly available. However, another model mentioned by EPA is the Multi-Chamber Concentration and Exposure Model (MCCEM). This model is available on EPA's exposure tools website,[32] but in a version (Windows 95 operating environment)[33] that will not run on currently available platforms. EPA should ensure that all the models it uses in its assessments are publicly available in a form that is accessible to the general public, complete with explanations on how to use the model and how the exposure endpoints are estimated.

2. EPA Should Provide Greater Clarity about How It Will Assess Consumer Exposures to Chemicals in Products.

The problem formulations for most of the 10 chemicals indicate that the chemical is found in either formulated products used by consumers or in articles with which consumers could come into contact. It is not clear how EPA will assess consumer exposures to these products. The exposure assessments must be able to estimate the consumer exposures from these chemicals based on whether they are found in formulated products or articles.

For chemicals that are primarily in articles, the approach and rationale for estimating consumer exposures should be described in detail because exposure assessments from articles are a new area of assessment. Industry and other stakeholders may not be familiar with the rationale and approaches used to estimate exposures from articles. The scientific basis for determining

[32] https://www.epa.gov/tsca-screening-tools/approaches-estimate-consumer-exposure-under-tsca
[33] https://www.epa.gov/tsca-screening-tools/multi-chamber-concentration-and-exposure-model-mccem-version-12

exposures from chemicals in articles must be established for the Agency to meet the statutory standard that requires TSCA risk assessments to quantify the likely (i.e., having a high probability of being true) duration, intensity, frequency, and number of exposures under the conditions of use. EPA should clearly identify the criteria for and scope of the tools chosen to be used in each circumstance.

For exposure assessments, EPA may need to make decisions about which products to focus on in the assessments among the various potential products in which the chemical may be found. To conduct the consumer exposure assessment, the assessor may need to focus on representative products in some of these use categories. The product types chosen to be used in the exposure models, the exposure routes, most relevant exposure scenarios, exposure endpoints, and rationale for the choices must be described. The greater the clarity and transparency of these explanations, the greater the likelihood the final assessment will be understood.

E. Use of Toxic Release Inventory (TRI) Data

EPA states in several of the problem formulations that TRI data will be used as a source of information on releases to the environment. TRI data may have a role to play as an element in chemical prioritization, but these data also have limitations. EPA states on the TRI website:

> The Toxics Release Inventory (TRI) provides data about environmental releases of toxic chemicals from industrial facilities throughout the United States, measured in pounds. **The quantity of releases, however, does not indicate the level of health risk posed by the chemicals.** Although TRI data can't tell you whether or to what extent you've been exposed to these chemicals, they can be used as a starting point in evaluating potential risks to human health and the environment.[34] (Emphasis added.)

EPA readily acknowledges in its *TRI National Analysis 2016: Releases of Chemicals* that "[h]uman health risk resulting from exposure to toxic chemicals are determined by many factors…"[35] These factors include environmental fate, individual exposures, chemical properties, and concentration, none of which are furnished through the TRI. For a chemical to present a risk, there must be a sufficient pathway and exposure, factors that TRI does not address. EPA should acknowledge and explain the limited value of TRI data in risk evaluation.

F. Use of Biomonitoring Data

Biomonitoring information is identified in several of the problem formulations as a type of data/information source for TSCA risk evaluations, but there is limited discussion of how or where it would be used. EPA should address in guidance the specific biomonitoring information it would rely upon in TSCA risk evaluations and how it would be used.

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[34] Taken from https://www.epa.gov/toxics-release-inventory-tri-program/tri-and-estimating-potential-risk.
[35] TRI National Analysis 2016, www.epa.gov/trinationalanalysis/, January 2018, Releases of Chemicals p.6.

Canada uses "biomonitoring equivalents" in its risk assessments under the Canadian Management Plan (CMP).[36] EPA should examine how those values, as well as Canada's assessments that are based upon them, might be used in the TSCA exposure assessments.

III. Hazard Assessment Considerations

A. Integrated Hazard Assessment and Use of Non-Animal Methods

It is important that a multidisciplinary review process, which integrates hazard information and data from *in vitro* and *in vivo* studies across different biological levels of organization for a given exposure scenario, be established for hazard evaluation, data review, and decision making contexts. Typically, this should be a transparent and structured analysis using the Bradford Hill causal considerations and, in particular, biological plausibility and empirical support (dose response, temporal concordance and consistency). The hazard information must be relevant to the specific exposure scenario and the integration of data should be applied initially for each data stream (epidemiology, *in vivo*, mechanistic) across similar types of study endpoints. The lines of evidence (human epidemiology, *in vivo* toxicity and mechanistic) must then be integrated using a transparent and objective approach.[37] Through such an integrated assessment, evaluators use the entire body of studies and the full weight of the scientific evidence. This approach avoids the pitfalls of selecting the lowest statistically significant finding of a response in a given study (as a default) without adequately framing the risk hypotheses and integrating data from different sources.

EPA states in the general response to comments on the initial 10 scope documents that it anticipates using data from alternative test methods for the risk evaluations.[38] This is consistent with the mandate under TSCA Section 4(h) to "reduce and replace, to the extent practicable, scientifically justified, and consistent with the policies of this title, the use of vertebrate animals in the testing of chemical substances or mixtures…"[39]

ACC supports EPA's continued efforts to identify, develop, and integrate new approach methodologies (NAMs) for regulatory decision-making according to the EPA OPPT *Strategic Plan to Promote the Development and Implementation of Alternative Test Methods*.[40] It is important that sufficient scientific confidence in each NAM be established for its intended application before use as a key piece of evidence in a hazard evaluation and limitations be acknowledged.[41] It is equally important that exposure information, at a fit-for-purpose level of resolution, is available to place these data into a risk context.

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[36] https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/human-biomonitoring-data-risk-assessment.html.
[37] Rhomberg, L.R., et al. 2013. A survey of frameworks for best practices in weight-of-evidence analyses. Critical Reviews in Toxicology, 43(9): 753-84.
[38] https://www.epa.gov/sites/production/files/2018-06/documents/rtc_10_pf_53118.pdf
[39] 15 U.S.C. § 2603 (h).
[40] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical
[41] *Ibid.*

B. Analysis of Mode of Action (MOA)

EPA acknowledges that it must further analyze the MOA for cancer risk in the problem formulations. ACC supports that analysis.

The Adverse Outcome Pathway (AOP) framework can be employed specifically as an organizing principle elucidating MOA and connections to adverse outcomes. The AOP framework is a tool to systematically organize available data and knowledge that describes scientifically plausible and causal relationships across multiple levels of biological organization between a molecular initiating event (MIE) and subsequent key events (KEs), culminating in an adverse outcome (AO) potentially relevant to risk assessment.[42] EPA researchers have been instrumental in developing AOPs and tools to facilitate the further development, review, and use of AOPs in scientific and regulatory endeavors.[43] Tools such as the AOP wiki can be mined for additional data and organizational principles as well as domains of applicability for various identified MOAs associated with chemicals.[44] Thus, whether evidence generally aligns or does not align with any proposed or known MOAs and/or AOPs should be a necessary consideration in integrating evidence to reach conclusions.

The Agency's focus on dose-response data and models reflects the fact that toxicology has evolved over the past 35 years from a largely observational field of study to a discipline that applies advanced scientific techniques and knowledge to investigate how chemicals interact with biological systems at the molecular, cellular, organ, and organism levels to understand the biological basis for the induction of toxicity. As a consequence of rapid advances in scientific understanding and the application of this knowledge to regulatory science policy and risk assessments, risk assessors can now evaluate biological events leading to toxicity and consider how, in a dose-response manner, these events relate to potential risks to human health. Despite the significant progress, movement away from default assumptions has been slow to occur, particularly in certain EPA programs. Failure to recognize and act on advances in scientific knowledge and the best available, most relevant scientific data and dose response models wastes significant research and development investments. It is also contrary to the TSCA Section 26 requirement that EPA rely upon best available science in science-based Section 6 decisions.

In its 2005 Cancer Guidelines, EPA is clear that when risk assessments are performed using only one set of procedures, it may be difficult for risk managers to determine how much health protection is built into a particular hazard determination or risk characterization.[45] EPA's Cancer Guidelines state:

> When there are alternative procedures having significant biological support, the Agency encourages assessments to be performed using these alternative procedures, if feasible, in order to shed light on the uncertainties in the assessment, recognizing that the Agency

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[42] Ankley, G.T., et al. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology & Chemistry, 29(3): 730-741. doi: 10.1002/etc.34.
[43] https://www.epa.gov/sites/production/files/2017-03/documents/aop_research_brief_03_2017.pdf
[44] https://aopwiki.org/aops
[45] US EPA, Guidelines for Carcinogen Assessment, Risk Assessment Forum, Washington, DC, EPA/630/P-03/001F, March 2005.

> may decide to give greater weight to one set of procedures than another in a specific assessment or management decision. [46]

In addition, the Agency says:

> If critical analysis of agent-specific information is consistent with one or more biologically based models as well as with the default option, the alternative models and the default option are both carried through the assessment and characterized for the risk manager. In this case, the default model not only fits the data, but also serves as a benchmark for comparison with other analyses. This case also highlights the importance of extensive experimentation to support a conclusion about mode of action, including addressing the issue of whether alternative modes of action are also plausible.[47]

EPA's Office of Pesticide Programs (OPP) has adopted the World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) MOA framework for organizing, evaluating, and integrating hazard and dose response information.[48] The same approach should be adopted for TSCA assessments. The MOA-framework can be used to illustrate the key events in a known toxicity pathway to address whether a reported statistically-significant response is consistent with what is expected based upon knowledge of the biological responses comprising the pathway. It should be noted that even if early biological responses/perturbations are detected, these observations are not necessarily adverse or precursors to adverse effects in living organisms because of adaptive or homeostatic mechanisms. To reliably predict toxicity, key events need to be causally linked to adversity with a clear understanding of dose response/temporal key event relationships. EPA should adopt and use the standard MOA templates for both cancer and non-cancer endpoints, such as the dose/temporal concordance and species concordance templates. These templates have been incorporated by the European Chemicals Agency (ECHA) in implementing Europe's REACH program.[49]

Because the scientific justification for assessing human relevance and selecting dose-response extrapolation methods for quantifying risks at environmentally relevant levels of exposure is highly dependent upon the determination of the likely operative MOA, the Agency should implement a uniform, systematic and explicit approach for evaluating a chemical dataset, using hypothesized MOAs and the evolved Bradford Hill causal considerations, to integrate evidence and derive weight of the evidence (WOE) confidence scores for potentially relevant MOAs.[50] This approach enables a side-by-side comparison of numerical WOE confidence scores for different hypothesized MOAs, including the default linear-no-threshold model, which permits better identification of the likely best MOA to use. The side-by-side quantitative MOA WOE confidence scoring method enhances transparency and improves communication amongst risk managers and the public. Furthermore, the best available science approach provides a

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[46] *Ibid,* at I-8.
[47] *Ibid*, at I-9.
[48] http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm
[49] https://echa.europa.eu/documents/10162/22315482/whoipcs_moa_template_withinstructions.docx/b98feba9-a37c-489d-94b0-dd5fbb2ed468
[50] Becker, R.A., et al. 2017. Quantitative weight of evidence to assess confidence in potential modes of action. Regulatory Toxicology & Pharmacology, 86: 205-220. doi: 10.1016/j.yrtph.2017.02.017

transparent, scientifically sound justification for using the most likely operative MOA as the basis for selecting the most appropriate extrapolation method that corresponds to that MOA to then calculate potential risks to humans for environmentally relevant exposures.

To illustrate this method, a case example has been developed based on data of rodent liver tumors induced by carbon tetrachloride (Attachment B). This case example used data and lines of evidence from previously published review articles, and relied on those authors' evaluations of the quality of the empirical evidence. Two hypothesized MOAs were evaluated: 1) induction of rodent liver tumors via a mutagenic MOA; and 2) induction of rodent liver tumors via a cytotoxicity MOA.

The quantitative MOA WOE confidence scoring results of this case example indicate:

- It is highly unlikely that carbon tetrachloride induces rodent liver tumors via a mutagenic MOA; and
- Cytotoxicity and sustained regenerative cellular proliferation is the likely operative MOA for induction of liver tumors in rodents by carbon tetrachloride; there are significant mechanistic data to support this non-linear, non-mutagenic MOA

Based on the comparison of quantitative MOA WOE confidence scores, there is strong scientific support for using a threshold extrapolation approach for evaluating the cancer risks of carbon tetrachloride. (In contrast, scientific justification is lacking to support a linear, no threshold extrapolation method for evaluating its cancer risks.)

Finally, another challenge in extrapolating animal data to human data involves having an understanding of the relative toxicokinetics. Significant strides have been made using physiologically based pharmacokinetic (PBPK) data and models in risk assessment to improve the accuracy of deriving dosimetry considerations.[51] However, it is important to recognize that some animal studies using conventional maximum tolerated doses (MTDs) are flawed and cannot be used to extrapolate to human doses because they exceed the kinetically-derived maximum dose (KMD). In a number of cases, substances show dose-dependent transitions in their mechanisms of toxicity.[52,53,54] This circumstance needs to be evaluated appropriately.

C. Ecological Hazard Characterization

EPA has used a simple approach to calculate the acute and chronic COCs, i.e., dividing the lowest study value by an assessment factor. Conservative, screening-level approaches, such as those utilized in the EPA's New Chemicals Program, can be appropriate to provide context at the problem formulation stage. However, in future scoping documents EPA should clarify the

[51] EPA discusses the need to evaluate available PBPK and empirical kinetic models for route-to-route and interspecies extrapolation of the point of departure (POD) in several of the problem formulations.
[52] Slikker, W., et al. 2004. Dose-dependent transitions in mechanisms of toxicity. Toxicology & Applied Pharmacology, 201(3): 203-25.
[53] Slikker, W., et al. 2004. Dose-dependent transitions in mechanisms of toxicity: case studies. Toxicology & Applied Pharmacology, 201(3): 226-94.
[54] Saghir S.A., et al. 2012. Assessment of diurnal systemic dose of agrochemicals in regulatory toxicity testing--an integrated approach without additional animal use. Regulatory Toxicology & Pharmacology, 63(2): 321-32.

circumstances under which further, higher-tier evaluation would be triggered, if necessary (e.g. species sensitivity distribution, etc.).

EPA should identify more sophisticated higher-tier approaches it may use for determining a hazard threshold, especially for data rich chemicals. Toxicity information, and when available, knowledge of mechanisms, are integrated with exposure-response models for risk-based environmental safety decision making. Within an environmental context, the assessment of safety does not end at the organism, but includes extrapolation to populations, communities, and ecosystems. For ecological risk assessment, the possibility of obtaining site-specific population data is a critical option for higher-tier assessment.

EPA should also consider the unique physico-chemical properties that can impact substances' pharmacokinetics and toxicity profiles, as well as their environmental fate and distribution.

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IV. Conclusion

ACC commends EPA on its efforts to gather the best available information for the problem formulation documents for the initial 10 chemicals undergoing risk evaluation under amended TSCA. EPA has demonstrated some screening-level assessment techniques that allow EPA to focus on the conditions of use that pose the greatest potential for risk. However, in situations where EPA may need to perform higher tier assessments for the risk evaluation, more guidance and information is needed on the types of data and techniques that EPA will utilize. This will enable industry to better understand how to provide EPA with the information it needs to perform high quality risk evaluations.

ATTACHMENT A

ATTACHMENT B

Attachment A

AMERICAN CHEMISTRY COUNCIL COMMENTS ON THE APPLICATION OF SYSTEMATIC REVIEW IN TSCA RISK EVALUATIONS

August 2018

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Executive Summary

The American Chemistry Council (ACC) appreciates the opportunity to provide comments on the U.S. Environmental Protection Agency (EPA) Office of Pollution Prevention and Toxics (OPPT) systematic review approach for chemical risk evaluations under the amendments to the Toxic Substances Control Act (TSCA).

ACC appreciates the effort for transparency and the progress toward documentation of the TSCA systematic review approach. However, there are some critical concepts and methodologies that remain to be discussed or fully developed in the current approach document. Following the consideration of initial comments received, and the further development of the approach in the draft risk evaluations for the first 10 chemicals, EPA should re-issue the systematic review document with updates and allow for additional review and stakeholder feedback.

ACC makes the following additional recommendations:

- **Data collection** – Within the data collection phase of systematic review, it is critical that EPA capture studies generated for regulatory purposes in order to be truly fit for purpose as part of the TSCA risk evaluation process.
- **Use of existing assessments** - EPA must conduct its own assessment of existing toxicity values as many are dated and were published without the benefit of systematic review. Doing so will enable EPA to consider available studies reflecting the best available science.
- **Study quality** - ACC appreciates EPA's intention to be highly transparent and consistent in its evaluations through the use of a quantitative scoring system. EPA should consider adding additional explanation of some of its proposed methods for study quality evaluation to increase transparency.
- **GLP -** EPA should ensure that the study quality evaluations retain consideration of the robust study designs and highly documented processes required for studies conducted using EPA/OECD test guidelines in accordance with GLP regulations.
- **Selection of key studies** - EPA should provide additional information highlighting how study quality and relevance will be considered in selection of key studies for derivation of toxicity criteria and in forming overall conclusions.
- **Mode of action (MOA) and mechanistic data** - EPA should add an explanation of the importance of incorporating information on MOA and mechanistic data in problem formulation.
- **Evidence Integration** - EPA should use a transparent process to integrate evidence that is standardized in such a way that ensures consistent use of best available science and weight of the evidence.

I. Introduction

On May 31, 2018, the United States Environmental Protection Agency's (EPA's) Office of Pollution Prevention and Toxics (OPPT) released the systematic review approach for chemical risk evaluations under the amendments to the Toxic Substances Control Act (TSCA).[1] The document is intended to outline the general principles guiding EPA's application of systematic review in the risk evaluations for the first 10 chemicals, as well as other chemicals that will be evaluated in the future. It is important that EPA's problem formulation documents be read in consideration of EPA's systematic review approach. ACC[2] has filed comments on the problem formulation documents for the first 10 chemicals in each of the dockets. These comments are attached and incorporated by reference.[3]

ACC appreciates the transparency and progress toward documentation of the TSCA systematic review approach. EPA has developed a strong baseline systematic review approach, emphasizing the importance of allowing for "fit-for-purpose" evaluations tailored to specific substances and an iterative evaluation process. The guidance outlined for data searches, data screening, and data extraction is comprehensive and useful. Notably, the current guidance has a strong focus on study quality, and thoroughly outlines the proposed steps for study quality evaluation for each domain of evidence.

However, there are some critical systematic review concepts and methodologies that remain to be discussed or fully developed in the current approach document, most notably for the process of evidence integration. Following the consideration of initial comments received, and the further development of the approach in the draft risk evaluations for the first 10 chemicals, EPA should re-issue the systematic review framework document with appropriate updates and allow for additional review and stakeholder feedback. In particular, at that time, EPA should put forward the standardized procedures the Agency will use for integrating evidence that ensures consistent use of best available science, weight of the scientific evidence, and, as applicable, an understanding of mode of action (MOA).

The systematic review process should have sufficient flexibility such that it can adapt to the realities of the chemicals being tested and the limitations in experimental methodology and laboratory techniques. For example, the challenges in collecting hazard, fate, and exposure data for chemicals with any one of a number of characteristics which make them "difficult

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[1] US EPA, Application of Systematic Review in TSCA Risk Evaluations (Final). Office of Chemical Safety and Pollution Prevention, Office of Pollution Prevention and Toxics, EPA Document # 740-P1-8001. Available at https://www.epa.gov/sites/production/files/2018-06/documents/final_application_of_sr_in_tsca_05-31-18.pdf

[2] The American Chemistry Council (ACC) represents the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier and safer. ACC is committed to improved environmental, health and safety performance through Responsible Care®; common sense advocacy designed to address major public policy issues; and health and environmental research and product testing. The business of chemistry is a $768 billion enterprise and a key element of the nation's economy. It is among the largest exporters in the nation, accounting for fourteen percent of all U.S. goods exports. Chemistry companies are among the largest investors in research and development. Safety and security have always been primary concerns of ACC members, and they have intensified their efforts, working closely with government agencies to improve security and to defend against any threat to the nation's critical infrastructure.

[3] See Attachment A.

substances" for testing purposes are well known. Results from common adaptations of typical test methods for difficult substances should not be blindly rejected but should be subject to expert judgment to confirm the validity and applicability of such data.

Below, we provide comments on the EPA systematic review approach, as well as specific suggestions on concepts and methodologies that EPA should consider as it continues to develop the TSCA systematic review process.

II. Problem Formulation and Data Collection

EPA begins its systematic review approach document by re-emphasizing TSCA's focus on clear problem formulation. The importance of problem formulation in systematic review is well documented and supported.[4,5] The objectives and relevant scientific question(s) for chemical hazard, exposure, and risk assessment are often complex and require thoughtful consideration prior to and throughout each evaluation. Further, the problem formulation step is vital for identifying critical concepts and potential issues that may be faced later in the risk evaluation process.

EPA should add discussion emphasizing the importance of incorporating information on MOA data in problem formulation, and consider organizing the problem formulation step around these data, even if the MOA is not entirely clear from the outset. Existing frameworks, such as the World Health Organization (WHO)/International Program on Chemical Safety (IPCS) MOA/Human Relevance (HR) Framework,[6,7,8,9] the Adverse Outcome Pathway (AOP) framework,[10] or other similar approaches may be useful.[11]

Within the problem formulation phase of the evaluation, EPA must clearly describe any decisions regarding its planned use of other EPA office or agency assessments of the chemical under review. Further, OPPT should not automatically adopt existing toxicity criteria in the absence of its own review and consideration of possible alternative values using the proposed

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[4] Rhomberg, LR, *et al.* 2013. "A survey of frameworks for best practices in weight-of-evidence analyses." Crit. Rev. Toxicol. 43(9):753-784. doi: 10.3109/10408444.2013.832727.
[5] National Research Council (NRC). 2014. "Review of EPA's Integrated Risk Information System (IRIS) Process." National Academies Press. 204p. Accessed on May 06, 2014 at http://www.nap.edu/ catalog.php?record_id=18764.
[6] Boobis, AR, *et al.* 2006. "IPCS framework for analyzing the relevance of a cancer mode of action for humans." Crit. Rev. Toxicol. 36(10):781-792.
[7] Boobis, AR, *et al.* 2008. "IPCS framework for analyzing the relevance of a noncancer mode of action for humans." Crit. Rev. Toxicol. 38(2):87-96.
[8] Meek, ME, *et al.* 2014. "New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis." J. Appl. Toxicol. 34(1):1-18. doi: 10.1002/jat.2949.
[9] Meek, ME, *et al.* 2003. "A framework for human relevance analysis of information on carcinogenic modes of action." Crit. Rev. Toxicol. 33(6):591-653.
[10] OECD Guidance document No. 184, Revised Guidance Document on Developing and Assessing Adverse Outcome Pathways. Available at http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en
[11] For example, see Borgert, CJ; Wise, K; Becker, RA. 2015. "Modernizing problem formulation for risk assessment necessitates articulation of mode of action." Regul. Toxicol. Pharmacol. 72(3):538-551. doi: 10.1016/j.yrtph.2015.04.018.

systematic review approach. We support EPA's intention, as specified in the problem formulation documents, to conduct its own independent assessment of existing toxicity values. In many cases, these existing reviews are dated and were published without the benefit of systematic review and consideration of available studies reflecting the best available science that have been more recently developed.

Regarding the data collection phase, the current approach for data searching, screening, and extraction is well developed. EPA provides detailed information on its plans to use specific search strategies and databases, how decisions will be made regarding screening (in both the abstract/title and full text screening phase), and how it will carry out the quality assurance (QA)/quality control (QC) process for all three parts of data collection. Further, EPA includes example search and screening strategies used for the first 10 chemicals, which provide helpful context on the implementation of this phase of the risk evaluation.

EPA's consideration of grey literature, such as technical reports, conference proceedings, and unpublished industry data, is well supported, as there are many sources that may be useful that have not been published in peer-reviewed journals. In order for this approach to be truly fit for purpose, it is critical that EPA capture studies generated for regulatory purposes at the data collection stage.[12] EPA should also consider the possibility of publication bias in the peer-reviewed literature; i.e., the possibility that studies with negative findings may not have been published.

ACC supports EPA's recommendation that the Agency pilot test the search and screening methods, which will be important for iterative evaluations. This will allow for changes to be made if it becomes clear that references have been missed by the use of specific search terms, or if relevant articles are being unintentionally screened out. Further, it is critical that EPA thoroughly describe the reasoning for any changes to risk evaluations resulting from pilot testing or other iterative phases of the assessment. Clarification is also needed as to how EPA will carry out iterative methods in later phases of an evaluation.

III. Data Evaluation and Integration

A. Application of Study Quality Criteria

Overall, the systematic review approach covers essential aspects of evaluating study quality. It indicates that EPA intends to thoroughly evaluate and fully consider the implications of the quality and relevance of the available evidence before incorporating it into its risk evaluations.

There are many positive attributes in the methods EPA describes, such as a training phase for reviewers to ensure consistency across quality evaluations. The specific criteria are informed by several existing, well-regarded evaluation systems that detail critical study quality and reporting criteria systems, such as the Strengthening the Reporting of Observational Studies in

[12] For example, OECD eChemPortal can serve as a resource: https://www.echemportal.org/echemportal/index.action

Epidemiology (STROBE) statement[13] and the Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument.[14]

The study quality evaluation process appears to be very time intensive, and it is unclear whether it is possible to complete it in full for every evidence type for each evaluation, given the tight regulatory deadlines under TSCA. It is also unclear whether, as an alternative, EPA may rely on existing quality evaluations, and, if so, how these evaluations will be evaluated to ensure they adequately fulfill the rigorous quality assessment requirements proposed for TSCA evaluations. Also, as discussed below, EPA should consider adding additional explanation of some of its proposed methods to increase transparency. As it is difficult to determine how some aspects of the study quality evaluation system will operate in practice, EPA should solicit and consider additional feedback on the performance of the study quality criteria approach as applied in the initial risk evaluations.

1. Evaluation Method

Page 33 of the systematic review approach states, "EPA/OPPT plans to use data with an overall quality level of High, Medium, or Low confidence to quantitatively or qualitatively support the risk evaluations, but does not plan to use data rated as Unacceptable." ACC agrees that unacceptable data should not be used in the risk evaluation. There is some concern that low confidence studies could be used to quantitatively support a risk evaluation. If there is low confidence in the study methods and/or reporting, then it should not be used to quantitatively support the derivation of a point of departure in a hazard assessment. Rather, it should be used qualitatively as a supporting study or in a weight-of-evidence determination for hazard characterization.

EPA states that it will not automatically assign lower confidence to studies not adhering to Good Laboratory Practice (GLP) or Organisation for Economic Co-operation and Development (OECD) guidelines, but rather, it will consider, "any and all available, relevant data and information that conform to the TSCA science standards" as acceptable.[15] What this might mean for academic studies, which are usually not conducted according to GLP requirements and may use non-standard methods, is unclear. EPA should ensure that the study quality evaluations retain consideration of the robust and highly documented process required by GLP guidelines, even if they are not GLP studies. As noted by Borgert et al., 2016, "…regulatory agencies have placed a high value on study reports that include sufficient detail to allow reanalysis of data to independently confirm results and support additional analysis using alternative methods of data evaluation."[16]

Borgert and co-authors also emphasize that GLP-compliance is much more than record keeping and reporting.

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[13] von Elm, E, *et al.* 2007. "Strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies." BMJ 335(7624):806-808.
[14] LaKind, JS, *et al.* 2014. "A proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument." Environ. Int. 73:195-207. doi: 10.1016/j.envint.2014.07.011.
[15] US EPA, Application of Systematic Review in TSCA Risk Evaluations (Final), at 32.
[16] Borgert, CJ, *et al.* 2016. "Does GLP enhance the quality of toxicological evidence for regulatory decisions?" Toxicol. Sci. 151: 206–213. Available at https://academic.oup.com/toxsci/article/151/2/206/2241188

> GLP requires justification of the test system and procedures; training certification and documentation for investigators and technicians involved in each scientific procedure; the measurement of a comprehensive set of study parameters, including: analytical characterization of test materials; analytical verification of concentration, stability, and homogeneity of dosing solutions; validation and calibration of instruments; adherence to standard operating procedures in conducting experimental activities; independent auditing of study procedures and data; adherence to animal welfare requirements; measurement of laboratory and animal room conditions, among many other important assurances of experimental quality. The goal of these requirements is to ensure that critical study parameters—parameters that go to the core of study quality—are, in fact, measured. Furthermore, GLP requires that a study protocol be selected and justified a priori, that any deviations from that protocol occurring during the study are documented with appropriate written explanation or justification, and that these elements are audited by an independent party before finalization of the study report. [...] In particular, GLP specifically meets their requirements for carefully recorded and audited data covering relevant exposure routes and doses.[17]

2. Scoring Method

Overall, the scoring examples shown are clearly and transparently laid out in a series of tables. The weighting scheme, metrics, and overall scoring are relatively straightforward. ACC appreciates EPA's intention to be highly transparent and consistent in its evaluations through the use of a quantitative study scoring system. However, the scoring system described in the current approach is complicated by many possible options that may or may not be used, such as weighting factors. This may result in very specific scores with a relatively narrow range, which may make interpreting studies of similar but not identical quality difficult (e.g., a score of 1 versus 1.7). Further, some of the weighting factors chosen involve substantial scientific judgment, and EPA should consider that some metrics may be more important to overall quality for specific studies, relative to others, indicating that a generic "one-size-fits-all" weighting factor could become problematic. For example, in the criteria for occupational exposure and release data evaluation, it is unclear why the metric of methodology in the reliability domain is given a weighting factor of 1, when other critical factors, such as reliability, are weighted at 2. Incorrect or inappropriate methodology could be just as critical of a flaw, if not more so, than some of the other metrics.

In addition, while the use of a 1-4 scale for judging whether a study is evaluated to have high confidence, medium confidence, low confidence, or be unacceptable for use is clearly laid out and justified, it is anticipated that there could be some confusion with the already much-used Klimisch system of study evaluation.[18] The Klimisch system is somewhat similar in that studies assigned a 1 or a 2 are considered reliable without restrictions, or reliable with restrictions, respectively. However, the Klimisch system differs from the one EPA is proposing by

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[17] *Ibid*, at 208.

[18] Klimisch, HJ, *et al.* 1997. "A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data." Regul. Toxicol. Pharmacol. 25:1-5.

attributing a score of 3 to studies that are not reliable, and a score of 4 designating a score is not assignable due to insufficient information. In other words, the scale used on EPA's approach is the opposite of the Klimisch system for scores of 3 and 4. Furthermore, Klimisch scoring does not use weights or calculate mathematical averages, but rather assigns qualitative overall integer values of 1, 2, 3, and 4. Since the Klimisch scoring is already broadly used in regulatory activities across the globe, EPA should consider harmonization for evaluating studies in order to avoid confusion and harmonize with other geographies.

3. Data Availability

The availability of data and other information required to verify and reproduce critical studies in the risk evaluation is also important. Any data that are used to derive toxicity criteria should be made publicly available to the greatest degree possible, while still protecting confidential business information (CBI) and other sensitive personal information, consistent with EPA's recently proposed rule on Strengthening Transparency in Regulatory Science.[19] This will facilitate transparency and allow others to consider and independently evaluate the quality, reliability, and interpretation of these data. For example, a frequent concern with published academic studies is that the data presented in either tabular or figurative form have already experienced some form of statistical transformation. In many cases, even an expert-level statistician cannot recreate the original data from these data.

Academic laboratories sometimes conduct their statistical analysis using laboratory personnel who are not professional statisticians. The technical issue with non-professional analysis is rarely whether the test was conducted correctly, but rather whether the most appropriate statistical test was selected. In a seminal study conducted by Begley and Ellis (2012), the study authors were unable to replicate the results from statistical analyses of 47 of 53 landmark pre-clinical cancer research papers.[20] This led to a flurry of other studies in different fields that have also reported similar findings. Thus, it is crucially important that data upon which regulatory actions are based be available for independent statistical analysis.

B. Evidence Integration

In the current systematic review approach document, the strategy for evidence integration lacks detail and specificity. Only general, high-level principles are described, and no specific weight-of-evidence methodology is presented as a baseline for TSCA assessments. EPA recognizes that the evidence integration phase of assessments is underdeveloped and indicates that it anticipates defining and demonstrating the process of integration in the forthcoming first 10 chemical draft risk evaluations. We expect that as EPA gains more experience with evidence integration, and can describe the standardized procedures the Agency will use for integrating evidence that ensures consistent use of best available science, weight of the scientific evidence, and, as applicable, understanding of MOA, the Agency will revise this guidance document. Such a

[19] See 83 Fed.Reg. 18768 (April 30, 2018). ACC has filed comments on this proposed rule at Docket ID: EPA-HQ-OA-2018-0259.

[20] Begley, CG; Ellis, LM. 2012. "Drug development: Raise standards for preclinical cancer research." Nature 483(7391):531-533. doi: 10.1038/483531a.

revision should include additional review and public comment. Below, we describe methods and concepts that EPA should consider as it further develops the approach to evidence integration.

1. General Methodology

First, EPA should use a transparent process to integrate evidence that is standardized in such a way to allow for greater efficiency. EPA should consider development of a structured narrative that fully describes how the different pieces of available evidence support a given conclusion/argument or an alternative.[21,22] In this way, EPA can clearly demonstrate how specific studies or data sources contributed to the final conclusion. This will ensure that the process by which EPA reaches conclusions about exposure, hazard, and/or risk will be well developed and transparent.

Second, as a part of the evidence integration narrative, EPA should clearly describe how the study quality evaluations will be used to weigh the evidence and reach conclusions for the different phases of the risk evaluation, including exposure assessments, hazard assessments, and any quantitative estimates of risk. For example, the current approach does not indicate whether a high-confidence study will always be given more weight than a medium-confidence study in formulating conclusions, or how other factors, such as study relevance, will be weighed with quality considerations. EPA should consider building from the published approaches for quantitative weight-of-evidence analysis, such as Bridges et al., 2017;[23] Becker et al., 2017;[24] and Dekant et al., 2017.[25]

Third, EPA should detail how it will conduct uncertainty analyses and communicate these uncertainties consistently and transparently in each risk evaluation.

2. Critical Concepts

While MOA/AOP evidence and mechanistic data are mentioned in several places in the systematic review approach, EPA should consider expanding its discussion of this important evidence, particularly in the evidence integration phase of evaluation. MOA/AOP evidence and mechanistic data should be weighed concurrently with observational and toxicology evidence and considered a critical organizing principle for the weight-of-evidence evaluation.

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[21] The National Academy of Sciences recommends templates for structured narrative justifications of the evidence-integration process and conclusions for the IRIS Program in the 2014 Review of EPA's Integrated Risk Information System (IRIS) Process, Committee to Review the IRIS Process; Board on Environmental Studies and Toxicology; Division on Earth and Life Studies; National Research Council, available at https://www.nap.edu/catalog/18764/review-of-epas-integrated-risk-information-system-iris-process.

[22] See also the guidelines for integrating evidence in Rhomberg, LR, *et al.* 2013. "A survey of frameworks for best practices in weight-of-evidence analyses." Crit. Rev. Toxicol. 43(9):753-784. doi: 10.3109/10408444.2013.832727.

[23] Bridges, J, *et al.* 2017. "Framework for the quantitative weight-of-evidence analysis of 'omics data for regulatory purposes." Regulatory Toxicology and Pharmacology 91 (2017) S46-S60.

[24] Dekant, W, *et al.* 2017. "A quantitative weight of evidence assessment of confidence in modes-of-action and their human relevance." Regulatory Toxicology and Pharmacology 90:51-71. doi: 10.1016/j.yrtph.2017.08.012.

[25] Becker, RA, *et al.* 2017. "Quantitative weight of evidence to assess confidence in potential modes of action." Regulatory Toxicology and Pharmacology 86:205-220.

The AOP framework can be employed specifically as an organizing principle that explains MOA and the connections to adverse outcomes. The AOP framework is a tool to systematically organize available data and knowledge that describes scientifically plausible and causal relationships across multiple levels of biological organization between a molecular initiating event (MIE) and subsequent key events (KEs), culminating in an adverse outcome (AO) potentially relevant to risk assessment.[26] EPA researchers have been instrumental in developing AOPs and tools to facilitate the further development, review, and use of AOPs in scientific and regulatory endeavors.[27] Tools such as the AOP wiki can be mined for additional data and organizational principles as well as domains of applicability for various identified MOAs associated with chemicals.[28] Thus, whether evidence generally aligns or does not align with any proposed or known MOAs and/or AOPs should be a necessary consideration in integrating evidence to reach conclusions.

Since the scientific justification for assessing human relevance and selecting dose-response extrapolation methods for quantifying potential cancer risks at environmentally relevant levels of exposure is highly dependent upon the determination of the likely operative MOA, the Agency should implement a systematic and explicit approach for evaluating a chemical dataset, using hypothesized MOAs and the evolved Bradford Hill causal considerations, to integrate evidence and derive weight of the evidence confidence scores for potentially relevant MOAs.[29] This approach enables a side-by-side comparison of numerical weight of the evidence confidence scores for different hypothesized MOAs, including the default linear no threshold model. This enhances transparency and improves communication among risk managers and the public. This best available science approach provides a transparent, scientifically sound justification for using the most likely operative MOA as the basis for selecting the most appropriate extrapolation method to then calculate potential risks to humans for environmentally relevant exposures.

In addition, EPA should describe how it will consider issues of the adversity of identified health effects when considering the weight of the evidence. For example, there may be animal studies that demonstrate statistically significant effects that are reversible, and/or epidemiology studies may show changes in blood biomarkers but are not predictive of clinical disease. Results of this nature (those for which the adversity or clinical relevance is either questionable or unclear) should be interpreted with caution when making causal conclusions regarding hazard, and when selecting endpoints for consideration as critical effects.

Finally, EPA should add a discussion of how it will consider questions of relevance in the data evidence integration and summary phases of the risk evaluation. EPA indicates that it will use a tiered approach to check for relevance at various points in each risk evaluation, including during data screening and selection. However, it is not entirely clear how data will be weighed according to relevance when integrating evidence to support conclusions when presumably, at

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[26] Ankley, GT, *et al.* 2010. "Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment." Environ. Toxicol. Chem. 29(3):730-741. doi: 10.1002/etc.34.
[27] https://www.epa.gov/sites/production/files/2017-03/documents/aop_research_brief_03_2017.pdf
[28] https://aopwiki.org/aops
[29] Becker, RA, *et al.* 2017. "Quantitative weight of evidence to assess confidence in potential modes of action." Regul. Toxicol. Pharmacol. 86:205-220.

this point in the evaluation, all evidence discussed was previously deemed relevant to the risk evaluation for some purpose.

3. Utilization of Existing Frameworks

EPA should consider reviewing and adapting portions of other established systematic review and weight-of-evidence frameworks. For example, one recent and generally well-developed framework is the European Food Safety Authority (EFSA) Guidance on the use of the weight-of-evidence approach in scientific assessments.[30] Critical concepts in weight-of-evidence are well described, including the consideration of relevance, reliability, and consistency within and across lines of evidence. Various options for causal frameworks are presented, and EFSA emphasizes that, in many cases, a single method often cannot cover all steps. Differing methods, or a combination of methods, may be needed for a given assessment. These fit-for-purpose decisions can be documented in the problem formulation phase of assessment and thus will be vetted via peer review and public comment.

4. Transparency

Transparency in the decision-making process is vital for producing scientifically defensible and understandable assessments. Clear, thorough discussions of all decisions will increase confidence and aid in the general acceptance of the findings and conclusions of TSCA risk evaluations. The transparency of overall conclusions on chemical hazard, exposure, and risk may also be enhanced by the use of tabular and/or graphical summaries of the weight-of-evidence conclusions. Further, it is important that in all phases of the assessment, but particularly in the evidence integration and summary sections of the assessment, EPA clearly describes all areas in which expert judgment was utilized.

IV. Conclusions

The EPA TSCA systematic review approach has many positive attributes, with several areas of guidance that have been very well developed. The approach focuses on fit-for-purpose evaluations and an iterative evaluation process, which allow for flexibility that is necessary given the wide array of chemical substances that will be reviewed under TSCA. However, there are several areas in the document that lack detail and specificity – most notably, evidence integration. In future iterations of the document and forthcoming risk evaluations, there should also be a more explicit discussion of several critical concepts, such as the use of MOA/AOP knowledge and mechanistic data and consideration of the adversity of effects.

In addition, it is unclear how the study quality evaluations will be fully incorporated into conclusions regarding hazard, exposure, and risk; the guidance document would benefit from additional discussion of its plans for this phase of analysis. Finally, it is critical that scientific judgment and iterative changes are transparent and justified in the final risk evaluations.

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[30] European Food Safety Authority (EFSA) Scientific Committee. 2017. "Guidance on the use of the weight of evidence approach in scientific assessments." EFSA J. 15(8):4971. doi: 10.2903/j.efsa.2017.4971.

Overall, the EPA OPPT systematic review approach would benefit from additional explicit guidance, which will increase transparency within the EPA TSCA systematic review approach and ensure objective, comprehensive, scientifically supported risk evaluations for TSCA chemicals. The ability to replicate EPA's analyses, based upon the detailed guidance, will increase public confidence in risk evaluations conducted by the agency.

Attachment B

IDENTIFYING THE LIKELY OPERATIVE MODE OF ACTION FOR CARBON TETRACHLORIDE INDUCED RODENT LIVER TUMORS

Scientific Causality Confidence Scores for Potential Modes of Action: Comparing the Weight of Evidence for a Mutagenic Mode of Action to a Threshold Cytotoxicity Mode of Action for Rodent Liver Tumors

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I. Executive Summary

Background: One of the critical elements of a chemical carcinogenic risk assessment is the determination of the likely operative mode of action (MOA). Determining the operative MOA by which a chemical can cause cancer is important for characterizing potential human health hazards and for selecting dose-response extrapolation methods for use in risk assessment at environmental levels of exposure. This case example focused on evaluating hypothesized MOAs involved in the induction of liver tumors in rodents by carbon tetrachloride.

This case example used the quantitative MOA WOE confidence scoring approach described in Becker et al., 2017[1]. This method provides a systematic and explicit approach for 1) evaluating a chemical dataset using hypothesized MOAs and the evolved Bradford Hill causal considerations (biological plausibility, essentiality, dose-response concordance, consistency, and analogy) and 2) deriving an overall confidence score for each hypothesized MOA. This enables a side-by-side comparison of numerical WOE confidence scores for each MOA, and the determination of which MOA is more likely to be operative.

Analysis and Results: Using the quantitative MOA WOE confidence scoring approach described in Becker et al., 2017 and data for carbon tetrachloride, we compared the WOE for a mutagenic MOA to the WOE for a threshold cytotoxicity MOA. We summarized the relevant dose-response and incidence data and developed WOE confidence scores for both a mutagenic MOA and a threshold cytotoxicity MOA (see sections III and IV for details).

This analysis indicates:

- It is highly unlikely that a mutagenic mode of action is plausible for carbon tetrachloride-induced rodent liver tumors. Based on the MOA confidence score of -36, the weight of evidence clearly does not support a mutagenic mode of action for carbon tetrachloride. The negative score indicates there is strong counter evidence for several of the early, diagnostic, KEs for a mutagenic MOA. In other words, the available data indicate that it is highly unlikely that rodent liver tumors are induced by carbon tetrachloride via a mutagenic mode of action

- The MOA causal confidence scoring results indicate that the more likely operative MOA is cytotoxicity and sustained regenerative cellular proliferation which exhibits a non-linear/threshold dose-response. Therefore, an increase in cancer risk would only occur at doses that exceed a specific threshold. There are significant mechanistic data to support a non-linear, non-genotoxic mode of action for carbon tetrachloride, and based on the MOA confidence score of +87, the weight of evidence clearly supports a cytotoxic mode of action for carbon tetrachloride induction of rodent liver tumors

Conclusions and Recommendations: Based on the weight of the evidence (indicated by comparison of the MOA confidence scores), the likely operative MOA is cytotoxicity, not mutagenicity. The overall pattern of observations is very consistent with a non-linear, threshold mode of carcinogenic action, as evident by the MOA confidence score of +87 for cytotoxicity compared to the mutagenic MOA confidence score of -36. Therefore, it would be inappropriate to use a linear default for extrapolating cancer risks. Instead, the causal weight of the scientific evidence analysis supports use of a threshold, non-linear method for determining potential cancer risks (i.e., an extrapolation method based upon cytotoxicity, for which cancer risk would only be operative at doses that exceed the threshold for induction of hepatic cytotoxicity).

[1] Becker RA et al., 2017. Quantitative weight of evidence to assess confidence in potential modes of action. Regul Toxicol Pharmacol. 86: 205-220. OPEN ACCESS: http://www.sciencedirect.com/science/article/pii/S0273230017300387?via%3Dihub

As the Agency moves forward with its problem formulation and risk assessment of carbon tetrachloride, we encourage you to consider use of the quantitative confidence scoring method for determining and communicating the more likely operative MOA based on the weight of scientific evidence. This method provides a scientifically based WOE approach for selecting the most appropriate extrapolation method for determining potential human health risks. In fact, this approach should prove to be useful for most, if not all, of the TSCA assessments now being developed by OPPT that deal with potential carcinogenic risks, especially where alternative (non-mutagenic) MOAs with supporting mechanistic data are credible.

II. Introduction

One of the critical elements of a chemical carcinogenic risk assessment is the determination of the likely operative mode of action (MOA). Understanding a chemical's mode of carcinogenic action forms the scientific basis for the selection of the dose-response extrapolation method that best aligns with the underlying biology of the specific MOA pathway, and subsequently, ensures that the best available science is used for quantifying potential cancer risks at environmental levels of exposures.

To improve transparency and objectivity in MOA analysis, the WHO/IPCS MOA framework has recently been extended using an approach that enables quantitative scoring of the confidence in the weight of the evidence of alternative hypothesized MOAs. We have attached the abstract and link to the open access, peer-reviewed publication detailing this quantitative method (Appendix A. Becker *et al.*, 2017). As described in the publication, a systematic and explicit approach is used for evaluating a chemical dataset using key events in the context of the evolved Bradford Hill causal considerations, in order to integrate evidence and derive confidence scores for potentially relevant MOAs. This enables a side-by-side comparison using numerical scores of scientific confidence in each hypothesized MOA, including a default mutagenic MOA, to better identify the more likely (i.e., best supported) operative MOA. We are in the process of developing several additional case examples on a variety of chemicals to further illustrate the application of the MOA quantitative confidence scoring method and to support the continued refinement of the method.

As EPA commences the risk assessment of carbon tetrachloride, we believe the application of the quantitative MOA confidence scoring method can, as part of EPA's consideration of best available science and weight of the evidence, inform and strengthen the Agency's problem formulation phase of the cancer risk assessment.

The quantitative MOA WOE confidence scoring approach detailed in Becker et al., 2017 is a systematic and explicit approach for evaluating a chemical dataset, using hypothesized MOAs and the evolved Bradford Hill causal considerations (biological plausibility, essentiality, dose-response concordance, consistency, and analogy), to integrate evidence and derive confidence scores for potentially relevant MOAs. The components consist of: 1) a set of defining questions for each of the Bradford Hill considerations coupled with a WOE rating and scoring procedure to guide data evaluation and WOE determinations; 2) a procedure to score the evolved Bradford Hill causal consideration for essentiality at MOA pathway level based on the highest score achieved by any one of the unique Key Events (KEs) in the pathway; 3) a technique for including the supporting evidence of later KEs, even though these are disease-specific and not diagnostic of a MOA for a particular chemical, by affording less evidentiary value to later KEs than the earlier, more MOA-specific KEs; 4) hierarchical weighting of the evolved Bradford Hill causality considerations; and 5) a straightforward arithmetic method to characterize the overall confidence score for each hypothesized MOA.

To document the application of the recently peer-reviewed quantitative MOA WOE confidence scoring approach (Becker et al., 2017), we have developed this case example using the published carbon tetrachloride rodent liver tumor MOA data. The steps of quantitative MOA WOE confidence scoring are discussed in detail

in Becker et al., 2017; they are briefly described here to help contextualize the case example tables presented in Sections III and IV.

- Step 1. Identification of postulated MOAs and KEs/KERs for the adverse outcome(s) of interest (See Section 2.1 of Becker et al., 2017). (Note: Steps 2 through 5 are conducted for each hypothesized MOA.)

- Step 2. Qualitatively evaluate the evidence in support of, or inconsistent with, the KEs/KERs (See Section 2.2 of Becker et al., 2017), using the evolved Bradford Hill causal considerations.

- Step 3. Quantitatively rate each KE/KER using the evolved Bradford Hill causal considerations (See Section 2.3.1 of Becker et al., 2017). In the qualitative and quantitative rating approach (Steps 2 and 3), the individual or series of KEs are evaluated against the defining question for each evolved Bradford Hill causal consideration, using the WOE rating categories to guide the determinations for scoring. The rating categories include strong (3), moderate (2), weak (1), no evidence (0), weak counter evidence (-1), moderate counter evidence (-2), and strong-counter evidence (-3).

- Step 4. Derive the composite score for each KE/KER by multiplying the quantitative rating score by the weight assigned for each of the evolved Bradford Hill causal considerations and adjust based on the MOA evidentiary value of each KE/KER ($\sum$ (weight $\times$ rate $\times$ evidentiary value) = KE/KER score) (See Section 2.3.2 of Becker et al., 2017). To derive the composite score, each Bradford Hill causal consideration has been given a numerical weight in accordance with their ranked importance, with a summed maximum of 100% (Section 2.3.1 of Becker et al., 2017). Essentiality of the KEs within the MOA is considered collectively since the interdependence of KEs is often illustrated through the impact of prevention or augmentation of an earlier KE on later KEs. Furthermore, all KEs/KERs are not necessarily weighted the same. This is because for a given adverse outcome, often the later KEs leading to the adverse outcome are the same for each of the hypothesized MOAs. These later KEs are often indicative of the disease process, whereas the earlier KEs are more chemical-specific and more influential in determination of MOA, so in this method the later KEs that are common across MOAs are assigned an evidentiary weighting value of 10%.

- Step 5. Integrate the evidence of causality for the MOA by calculating the sum of the scores for all KEs/KERs and then dividing by the total maximum score to derive the "MOA confidence score" (See Section 2.4 of Becker et al., 2017). To calculate the overall WOE confidence score for a hypothesized MOA, KE scores are summed and normalized by dividing by the maximum possible score and then multiplying by 100. This simple normalization procedure allows for comparison of quantitative confidence scores in cases where the number of KEs differs between hypothesized MOAs. Total scores may be negative if, for a hypothesized MOA, there is strong counter evidence for several of the early, most diagnostic KEs.

- Step 6. Compare the quantitative confidence scores for the hypothesized MOAs, and select the MOA for which confidence in the supporting data is highest (See Section 2.4 of Becker et al., 2017).

Using the quantitative MOA WOE confidence scoring approach, in Sections III and IV below, we have summarized the relevant dose-response and incidence data and developed WOE confidence scores for a mutagenic MOA and for a threshold cytotoxicity MOA.

Note: The intent of this case study is to illustrate the quantitative scoring methodology. It is not intended to be a complete discussion of all available and relevant studies. To that end, we did not conduct an in-depth systematic review of the available literature, but we based this evaluation on data and lines of evidence from already published review articles, and those authors' evaluations of the quality of the empirical evidence. The data and lines of evidence used in developing this case example are from scientifically peer reviewed and journal published articles. For the development of this specific case example, the primary authors (LHP and MKM) developed the initial evaluation and an additional MOA expert (RAB) provided peer review of the interpretation and quantification of the MOA scores. As indicated in Becker et al., 2017, with respect to biological plausibility of hypothesized modes of action leading to cancer, both the mutagenic mode of action and the cytotoxic/regenerative cell proliferation mode of action are considered to be well documented, and thus the biological plausibility of these MOAs are not considered further herein. Rather, chemical-specific supporting data for the evolved Bradford Hill causal considerations (essentiality, dose-response, incidence and temporal concordance, consistency, and analogy) are specifically evaluated for the Key Events/Key Event Relationships (KEs/KERs) as a basis to apply the quantitative weight of evidence confidence scoring method.

Acknowledgments: This project was partly supported by contributions from ACC's Science and Research Division and from ACC's Center for Advancing Risk Assessment Science and Policy.

III. Evaluating the WOE for a Mutagenic MOA

Carbon Tetrachloride (CTC) has been investigated extensively over many years, both *in vitro* and *in vivo*, for evidence of genotoxicity/mutagenicity, in an attempt to demonstrate a key role for a DNA-reactive mode of action (MOA). The key events for a mutagenic MOA leading to hepatocellular carcinoma, identified based on a different chemical (Moore *et al.*, submitted), would be as follows for CTC:

1) Metabolism to a reactive intermediate leading to the formation of pro-mutagenic CTC-specific DNA adducts
2) Insufficient repair or mis-repair of pro-mutagenic CTC-DNA adducts
3) Early induced mutation in cancer critical genes in target tissue
4) Cellular proliferation, clonal expansion of mutant cells, and progression
5) Hepatocellular carcinoma (HCC)

Given the large number of genotoxicity/mutagenicity datasets for CTC, it is normal to expect a mix of positive (genotoxic/mutagenic) and negative results. The available relevant data on CTC genotoxicity are summarized in two key publications (Manibusan *et al.,* 2007; Eastmond *et al.,* 2008) and the 2005 ATSDR Toxicological Profile for CTC, and the information described here stems mainly from those summary sources. A more recent publication, Uehara *et al.*, 2013, provides supporting data for the later KEs (#4 & 5), which are considered converging KEs, ones which intersect with other AOPs & MOAs, all leading to HCC. While there is some evidence for the later key events for a mutagenic MOA for CTC-induced HCC, for the early, influential key events—which are critical to a determination of a mutagenic MOA-- there are either no data or only sparse supporting data (typically with explanations limiting their relevance), and a significant amount of counterfactual data.

These proposed key events for a mutagenic MOA for CTC are shown schematically in Figure 1-1:

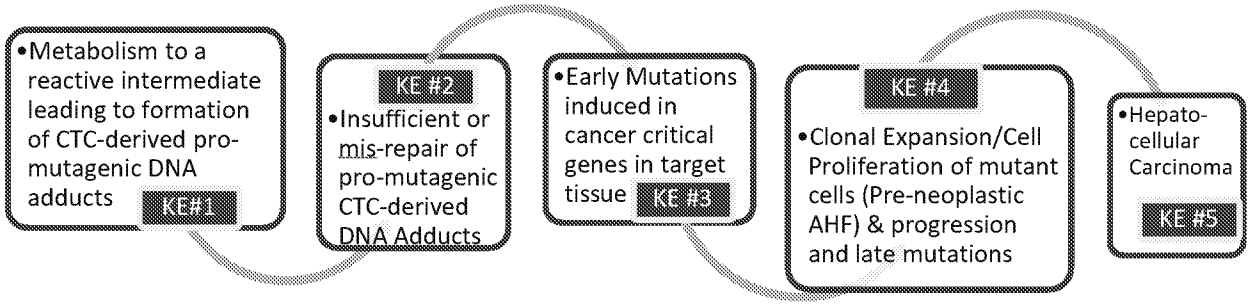

Figure 1-1. Postulated Mutagenic Mode of Action for Carbon Tetrachloride

Carbon tetrachloride (CTC) is metabolized to the highly reactive trichloromethyl free radical by hepatic CYP2E1 metabolism, which can bind macromolecules and lead to: Key Event #1) Formation of pro-mutagenic CTC-derived DNA adducts. This would be followed by Key Event #2) Insufficient or mis-repair of the pro-mutagenic CTC-derived DNA adducts, resulting in Key Event #3) Formation of early mutations induced in cancer critical genes in target tissue, resulting in Key Event #4) Cellular proliferation and clonal expansion to form pre-neoplastic, Altered Hepatic Foci (AHF), along with progression over time (including additional mutations and cell proliferation) to result in Key Event #5) Hepatocellular carcinoma (HCC), the presence of liver tumors.

A. Qualitative Evaluation of the Weight of Evidence (WOE) for Carbon Tetrachloride (CTC) Acting *via* a Mutagenic MOA (Manibusan *et al.*, 2007; Eastmond *et al.*, 2008; others)

Table 1-1. Qualitatively Evaluate the Comparative Weight of Evidence for Carbon Tetrachloride (CTC) Acting *via* The Mutagenic MOA (Manibusan *et al.*, 2007; Eastmond *et al.*, 2008; others) – (Step 2).

Key Event	Supporting Data	(Potentially) Inconsistent	References
KE#1 Metabolism of CTC to a reactive intermediate that leads to the formation of CTC - induced pro-mutagenic DNA adducts	Use of CYP2E1 knockout mice resulted in no liver histopathological effects, but this is not sufficient to demonstrate essentiality of KE#1 - the formation of CTC-induced pro-mutagenic DNA adducts. There are very limited *in vivo* data to demonstrate DNA binding: either ^{14}C or lipid peroxidation or oxidative adducts, and no data for CTC-derived adducts or Mass Spectrometry support, or evidence of DNA damage, except only in presence of significant cytotoxicity. No CTC–DNA adduct identification has been made, while the polar adducts observed in Syrian hamster liver DNA appear to be derived from lipid peroxidation products (Wang & Liehr, 1995).	Most CTC *in vivo* datasets on DNA binding/DNA damage (36 data sets) are negative or equivocal; DNA adducts are from lipid peroxidation, oxidative adducts, or ^{14}C-binding with no Mass Spectrometry info, thus no data on CTC-induced pro-mutagenic adducts. CTC metabolism resulting in terminal incorporation into cell 1-carbon pool metabolism cannot be ruled out. No ↑ in 8-oxo-dG adducts in liver or in liver mRNA for *Ogg-1*, *Apex*, or *polß*—all DNA-repair genes (later time points).	**CYP2E1 activating metabolism:** Wong *et al.*, 1998. **Neg strand breaks:** Schwarz *et al.*, 1979; Stewart, 1981;Bermudez *et al.*, 1982; Kitta *et al.*, 1982; Barbin *et al.*, 1983; Brambilla *et al.*, 1983 + **DSB w/ sig hepatotox:** Gans & Korson, 1984; Sasaki *et al.*, 1998; Cabre *et al.*,1999 **Oxidative/lipid peroxidation adducts:** Hadley and Draper, 1990; Chaudhary *et al.*, 1994; Takahashi *et al.*, 1998; Chung *et al.*, 2000; Wacker *et al.*, 2001; Kadiiska *et al.*, 2005; Lopez- Diazguerrero *et al.*, 2005 **Negative genomics and no oxidative DNA adducts**: Uehara *et al.*, 2013 **No CTC-DNA adduct:** Wang & Liehr
KE#2 Insufficient or mis-repair of CTC-induced DNA Adducts	No data available with only two flawed UDS datasets that indicate induction of DNA replication—both associated with significant hepatotoxicity.	Several negative UDS datasets with CTC; ↓ *Polß* and unchanged *Apex, & Ogg-1* mRNA in liver neonatal mouse model with CTC vs control.	**Negative UDS**: Butterworth *et al.*, 1989; Selden *et al.*, 1994; Craddock and Henderson,1978; Mirsalis & Butterworth, 1980; Mirsalis *et al.*, 1982; Mirsalis, 1987; Madle *et al.*,1994 **Positive (flawed) UDS**: Craddock & Henderson, 1978; Ikegwuonu & Mehendale, 1991 **DNA-repair mRNA:** Uehara *et al.*, 2013
KE#3 Early Mutations induced in cancer critical genes	No gene mutation data in cancer critical genes. Many *in vitro* positive mutation data; but very few *in vivo* positive genetox assays, and they are flawed: MN—only with significant hepatotoxicity)	Three negative *in vivo* transgenic mouse mutation assays; several negative *in vivo* MN/chromo datasets	**Negative TGRAs**: Mirsalis *et al.*, 1994; Tombolan *et al.*, 1999; Hachiya and Motohashi, 2000; Lambert *et al.*, 2005 **Positive (flawed) MN:** Van Goethem *et al.*, 1993, 1995 **Negative MN/Chromo:** Curtis and Tilley, 1968; Sawada *et al.*, 1991; Uryvaeva & Delone, 1995

Key Event	Supporting Data	(Potentially) Inconsistent	References
KE#4 Clonal Expansion/Cell Proliferation to form Pre-neoplastic AHF with	**40 mkd CTC: regenerative cellular proliferation, ↑ BrdU-cells in peri-portal zone at 24 & 36 hrs; plateauing at 48 hrs.* AHF in high dose mice & rats 13-wk CTC inhalation tox	No data available	Glende *et al.*, 1986; Glende *et al.*, 1992; Lee *et al.*, 1998; Eschen-brenner *et al.*, 1946; Nakata *et al.*, 1975; Doolittle *et al.*, 1987 AHF: Nagano *et al.*, 2007
progression and late mutations	Neonatal mouse: 9-14 wk of 0.2 ml/kg CTC (ip; 2x/wk): liver foci, ↑ BrdU-positive cells; ↑ apoptosis; ↑ oxstress markers: 4-HNE, anisonucleosis, lipofuscin granules		**CTC-induced liver foci, oxidative stress, cell proliferation, apoptosis:** Uehara *et al.*, 2013.
KE#5 Hepatocellular Carcinoma	*Increased incidence of liver tumors in both rats & mice. HCC appears only at the high dose in rats and mid & high doses in mice* In a neonatal mouse model with heightened sensitivity and specificity for tumors: ↑ adenomas & carcinomas in mouse liver w/ CTC were reported.	No data available	Adervont, 1958; Edwards and Dalton, 1942; Edwards *et al.*, 1942; Della *et al.*, 1961; JBRC *et al.*, 1998 **HCC neonatal mouse:** Uehara *et al.*, 2013

**Italicized* entries same as late, converged KEs for CTC cytotoxic/regeneration MOA analysis.

Table 1-2. Incidence of Tumors in F-344 rats and BDF1 mice exposed to carbon tetrachloride vapor for 104 weeks (6hrs/day, 5 days/week). (JBRC *et al.*, 1998)

Tumor	F-344 rats							
	MALE				FEMALE			
	0	5 ppm	25 ppm	125 ppm	**0**	**5 ppm**	**25 ppm**	125 ppm
Adenoma	0/50	1/50	1/50	21/50	0/50	0/50	0/50	40/50
Carcinoma	1/50	0/50	0/50	32/50	0/50	0/50	3/50	15/50
Adenoma or Carcinoma	1/50	1/50	1/50	40/50	0/50	0/50	3/50	44/50
	BDF1 mice							
Adenoma	9/50	9/50	27/50	16/50	2/50	8/49	17/50	5/49
Carcinoma	17/50	12/50	42/50	48/50	2/50	1/49	33/50	48/49
Adenoma or Carcinoma	24/50	20/50	48/50	50/50	4/50	9/49	44/50	48/49

Empirical Support Dose- and Temporal-Concordance:

Most of the available *in vivo* data pertinent to early key events are negative, and cannot support dose-response or temporal concordance for these diagnostic key events for a mutagenic MOA. Later key events, which are converged with other alternative MOAs, have good support because they are consistent with the biological progression for liver tumors.

KE#1 Good supporting data for the initial metabolism step based on the CYP2E1 knock-out mice study demonstrating no liver toxicity, but these data are not sufficient to demonstrate the subsequent formation of pro-mutagenic CTC-derived DNA adducts, which is critical for KE#1. Although DNA binding has been reported for *in vitro* experiments with naked DNA, there is no mass spectrometry-determined demonstration of *in vivo* CTC-derived DNA adducts. The only potential weak *in vivo* support comes from ^{14}C-radiolabeled data that are

ambiguous as to whether they represent adducts or incorporation of radiolabel into normal nucleotides. It is noted that CTC metabolism resulting in incorporation of CTC-derived radiolabeled carbon into cell 1-carbon pool metabolism cannot be ruled out. No CTC–DNA adduct mass spectrometric identification has been made, while the polar adducts observed in Syrian hamster liver DNA appear to be derived from lipid peroxidation products (Wang & Liehr, 1995).

KE#2: Some unreliable, flawed, liver unscheduled DNA synthesis (UDS) positive data are confounded with high hepatotoxicity; several datasets demonstrate *in vivo* negative CTC-liver UDS; Decreased (*Polβ)* or unchanged (*Ogg-1, & Apex)* mRNA (DNA repair-related genes) were quantified in mouse liver with CTC (Uehara *et al.*, 2013).

KE#3: Available *in vivo* evidence is either counterfactual (no increased mutations in 3 *in vivo* transgenic rodent assays) or critically flawed and unreliable due to concurrent high hepatotoxicity.

KE#4: AHF are present in dose-responsive manner (only at two highest exposure levels) after 13-wk exposures to CTC in mice and rats (Nagano *et al.*, 2007). Reasonable support for temporal concordance for effects related to biological progression in CTC-treated mice described in Uehara *et al.,* 2013.

Progression-related events increased with CTC treatment over time (9 wks vs 14 wks): Foci: 0% vs. 12.5%; oxidative damage: 4-HNE; Cell proliferation (BrdU uptake) 15% vs 42%; apoptosis (TUNEL staining).

However, 8-oxodG adducts, which would represent secondary DNA damage (not direct-binding of CTC –derived metabolites to form pro-mutagenic CTC-derived adduct), were not increased by CTC compared to controls, with a non-statistically significant increase in CTC-treated mice at 9 wks which disappeared in the 14-wk treatment group and was described as not increased by the authors (Uehara *et al.*, 2013). Expression of DNA-repair related gene *Polβ* mRNA was lower in CTC-treated 9- and 14-week-old mice compared with controls, while mRNA for *Ogg1* and *Apex* was not changed. These results do not support DNA damage directly from CTC exposure and thus do not support a mutagenic MOA for CTC-induced HCC.

KE#5: Many datasets consistently demonstrate dose-response and temporal concordance for CTC-induced HCC, both within and across studies. Increased incidence of liver tumors was reported in both rats and mice (see Table 2). Hepatocellular carcinomas appear only at the high dose in rats and mid and high doses in mice.

In a neonatal mouse model, with heightened sensitivity, adenomas & carcinomas only appeared at the end of the study, at 22 wks (14 wk of CTC), but were not present at 17 wks (9 wks of CTC), while other early precursor key events occurred before tumor formation, also demonstrating temporal concordance (Uehara *et al.,* 2013).

Table 1-3. Dose and Temporal Concordance Table

Temporal Concordance →

Dose Concordance ↓

Temporal	<24hrs.	12-36 hrs.	24-72 hrs.	48 hrs.-months	2 yrs. Cancer studies
Dose/Conc.	Metabolism to rxt intermed. Leading to the formation of pro-mutagenic DNA adducts (KE #1)	Insufficient repair or mis- repair of pro- mutagenic DNA adducts (KE#2)	Early induced mutation in cancer critical genes (KE #3)	Cell prolifer'n, clonal expansion of mutant cells, & progression (KE#4)	Hepatocellular carcinoma (HCC) (KE #5)
0 ppm					
5 ppm					
20 ppm				+ (mice)	
25 ppm					++ (mice)
100 ppm				++ (mice)	
125 ppm					+++ (rats & mice)
270/810 ppm^				+++ (rats & mice)	
Mouse*:oral, 1,000; 2,000 Mkd		+/- [UDS-signif. hepatotox or hydroxyurea]			
Hamster, Rat**	[Lipid peroxide-ation/oxidation adducts; **not MS CTC-derived adducts**]				
Rat, Mouse, Hamster***	+/- [^{14}C-DNA binding; no MS]				
Rat MN#			+/- [Surrogate: MN w/ signif. hepatotox]		
Neonatal Mouse&				9 Wks of CTC Liver foci: 0% BrdU+ cells: 15% Adenomas 0% Carcinomas 0% [+ (neonatal mice)]	14 Wks of CTC Liver foci 12.5% BrdU + cells 42% Adenomas 10% Carcinomas 25% [++ (neonatal mice)]

+++ = strong evidence; ++ = moderate evidence; + = weak evidence +/- = equivocal.

^AHF present in mice and rats after 13-wk exposure to CTC at two highest exposures only (270 & 810 ppm): Nagano *et al.,* 2007

* UDS: Craddock & Henderson, 1978; positive attributed to tissue regeneration (not DNA damage); Ikegwuonu & Mehendale, 1991: positive attributed to use of hydroxyurea in experimental design as inadequate DNA replication inhibition.

** Hamster: Wang & Liehr, 1995 (Hamster); Rat: Wacker *et al.,* 2001; Chung *et al.,* 2001; Chaudhary *et al.*, 1994.

***Castro *et al.*, 1989; Animals injected with 770 mg/kg radiolabeled CTC 6 hours before sacrifice; no MS to structurally identify CTC-induced pro-mutagenic adducts (could be 1-C pool incorporation).

#Van Goethem *et al.*, 1993, 1995: MN increase measured during wave of cell proliferation after extensive liver toxicity.

&Uehara *et al.*, 2013: 8-wk mice ip CTC (0.2 ml/kg; 2x/wk; 9 & 14 wk CTC treatment) cell proliferation, oxidative damage at 14 wk; increased foci, cell proliferation, oxidative damage, and liver tumors at 14 wk; decreased *Polß*, and unchanged *Ogg-1* and *Apex* mRNA in liver at 9 & 14 wks CTC; ~35% liver tumors (adenomas + carcinomas) at 14 wks.

B. Evolving Bradford Hill Causal Considerations: Qualitative and Quantitative Data Evaluation

Qualitative and quantitative rating categories. [See Becker et al. 2017 for details]

Qualitative	Quantitative	Category description
Strong	3	Multiple studies and/or extensive data provide convincing evidence that the substance causes the KE.
Moderate	2	Some evidence (direct or indirect) indicating the substance causes the KE, but scientific understanding is not yet completely established. There may be some studies that are equivocal.
Weak	1	Very limited evidence (direct or indirect) that the substance causes the KE along this pathway. Scientific understanding of the KE is limited.
No Evidence	0	No data available to support or negate causation of this KE by the substance.
Weak Counter	−1	There is very limited contradictory evidence (direct or indirect) that the substance does not cause this KE.
Moderate Counter	−2	Some evidence (direct or indirect) indicating that the KE is not caused by the substance, but scientific understanding is not completely established. There may be some studies that are equivocal.
Strong Counter	−3	Multiple studies and/or extensive data provide convincing evidence that the substance does not cause this KE.

Biological Plausibility—While the biological plausibility of CTC acting *via* a mutagenic MOA is reasonable, and there are *in vitro* data demonstrating *in vitro* mutagenic activity, there are no supporting *in vivo* data beyond activation of CTC to a reactive metabolite, with substantial counterfactual data to indicate that there is no reliable indication of *in vivo* mutagenicity from CTC.
Qualitative Rating for Biological Plausibility – Strong Counter Evidence for mutagenic MOA (-3)

Essentiality – Essentiality was demonstrated for metabolism and formation of the reactive metabolite by Wong *et al.,* (1998) that showed CTC-induced hepatotoxicity in mice was blocked by using CYP2E1 knockout mice (cyp2e1 -/-); exposure to 1.59 g/kg CTC *via* ip injection resulted in no increase in AST or serum ALT, and an absence of liver histopathology. However, no other reliable data were identified to address Essentiality for a mutagenic MOA for CTC-induced HCC based on influential early key events. No CTC–DNA adduct identification has been made, while the polar adducts observed in Syrian hamster liver DNA appear to be derived from lipid peroxidation products (Wang & Liehr, 1995).

There is some support for essentiality for the later key events, with data showing CTC-induced foci formation can be inhibited by treatment with anti-oxidants (yam) (Chan *et al.,* 2010), which assumes that decreased incidence of foci results in a corresponding decrease in HCC incidence. This is not support for a mutagenic MOA, which relies on the early influential KEs, but could support the link between foci (KE#4) and HCC (KE#5), generally.
Qualitative rating for Essentiality for a Mutagenic MOA: No Evidence (0)

Empirical Support (Dose and Incidence Concordance) - Effects/KEs/Surrogates measured did increase with *in vitro* dose (KEs#1 and 3), but either no evidence or only counter-evidence for *in vivo* effects of early KEs (KE#2: negative UDS or flawed and unreliable positive UDS and no increased mRNA for several DNA-repair genes; KE#3: three negative transgenic *in vivo* mutation assays and several negative MN/CA assays in rodents). Later KEs (#4 & 5) do show dose- response and incidence concordance, but these key events are less informative as they are not specific for a mutagenic MOA, which is largely determined by earlier KEs (#1-3).

KE#1: Metabolism to reactive metabolite leading to the formation of pro-mutagenic CTC-DNA adducts. While the data

supporting metabolism of CTC *via* CYP2E1 to the trichloromethyl peroxy radical are available, data on subsequent formation of pro-mutagenic CTC-derived adducts are not available. Direct evidence is lacking and the indirect evidence is not convincing. Although there is evidence for *in vitro* binding to naked DNA, appropriate *in vivo* data are lacking. The ^{14}C-DNA binding *in vivo* could be from incorporation of radiolabeled CTC into 1-carbon metabolism; there is no mass spectrometry data to support direct binding of CTC-derived metabolites to DNA to form adducts; the evidence for oxidative damage & peroxidative damage is strong, but does not address CTC-derived pro-mutagenic adducts. In fact, no CTC–DNA adduct identification has been made, while the polar adducts observed in Syrian hamster liver DNA appear to be derived from lipid peroxidation products (Wang & Liehr, 1995). Indeed, most of the DNA damage-related datasets for CTC (36 datasets) are negative or equivocal, providing moderate (but not definitive) counter-evidence.
KE#1 Qualitative Rating – Weak Counter Evidence (-1) for the formation of pro-mutagenic CTC-derived adducts.

KE#2: There are no reliable data to support (the only positive study is an unreliable UDS with significant hepatotoxicity) and some data to refute, with 7 negative UDS datasets; more recently no change or decreased mRNA for 3 DNA repair genes in CTC- exposed (14 wks) mouse liver (Uehara *et al.*, 2013). The neonatal mouse assay has a heightened sensitivity and specificity for tumor response (McClain *et al.,* 2001) and tumors develop rapidly so tumors evident at week 22 are not directly comparable to those reported in traditional 2-year, longer term rodent chronic studies.
KE#2 Qualitative Rating – Moderate Counter Evidence for mutagenic MOA (-2)

KE#3: There are *in vitro* positive datasets to support KE# 3 early induced mutation in cancer critical genes, but no reliable *in vivo* data to support this KE (flawed *in vivo* MN with significant hepatotoxicity), and there are several in *vivo* datasets to counter a mutagenic effect *in vivo* from CTC, with 3 negative transgenic *in vivo* mutation studies (rat & mouse) and several negative *in vivo* chromosomal effect studies (MN and CA).
KE#3 Qualitative Rating – Strong Counter Evidence for mutagenic MOA (-3)

KEs (#4 & #5): These key events are converging ones, at/after intersection points with other alternative MOAs; thus, although strongly supported with empirical data (13-wk CTC AHF (Nagano *et al.*, 2007); sensitive neonatal mouse data (Uehara *et al.*, 2013), these KEs are of lesser importance in the overall determination of a mutagenic MOA.
Qualitative Rating –Moderate (2) for KE#4 & Strong (3) for KE#5 empirical support; but these key events are not exclusive to mutagenic MOA.

Empirical Support (Temporal Concordance)

KE#1: While reactive metabolites must & do occur (substantiated by the CYP2E1 KO lack of liver tumors), the formation of pro-mutagenic DNA adducts has not been demonstrated considering the negative/equivocal DNA damage data. This KE is the starting point for the sequence of KEs for a mutagenic MOA, but there is no definitive *in vivo* evidence of CTC- induced DNA adducts. No CTC–DNA adduct identification has been made, while the polar adducts observed in Syrian hamster liver DNA appear to be derived from lipid peroxidation products (Wang & Liehr, 1995). The mostly negative/equivocal data on DNA damage provides weak counter evidence.
KE#1 Qualitative Rating – Weak Counter Evidence for Mutagenic MOA (-1)

KEs#2 & 3: Very little supportive *in vivo* evidence and substantial *in vivo* counter evidence for both KE#2 (negative UDS; mRNA unchanged/decreased for DNA repair genes) and for influential KE#3, which renders temporal concordance analysis somewhat moot. If indeed CTC operates *via* a mutagenic MOA, then KEs#1-3 must occur early, but *in vivo* evidence for KE#3 includes all counter evidence. Counter evidence from no increased mRNA for DNA repair genes is from later timeframe (14-wk CTC dosing) so does not offer support/counter evidence for temporal concordance.
KE#2 and 3 Qualitative Rating –Moderate Counter Evidence (-2) & Strong Counter Evidence (-3) for KEs#2 & 3 for Mutagenic MOA

KEs#4 &5: These are supported *vis-à-vis* temporal concordance, with cell proliferation increased with increasing duration of treatment; and foci and HCC identified only at study termination, increased cell proliferation at week 9 and increased incidence of liver foci and adenomas and carcinomas at week 14 with the sensitive neonatal mouse model

(Uehara *et al.*, 2013). Presence of AHF without adenoma or carcinoma (Nagano *et al.*, 2007) supports temporal sequence of late KEs.

Qualitative Rating –Moderate (2) for KE#4 & Strong (3) for KE#5 empirical support; not exclusive to Mutagenic MOA

Consistency & Analogy

Other haloalkanes are predicted to have the potential to cause cancer, but not necessarily *via* a mutagenic MOA. Indeed, a recent publication on methylene chloride (dichloromethane; DCM)—a close structural analogue to CTC and a mouse liver carcinogen--found no changes in gene expression in any genes related to DNA repair following a 13-wk exposure to mice, even at high (4000 ppm) exposures, a very strong indication that there was no genotoxicity response (Andersen *et al.*, 2017). DCM is also metabolized *via* CYP2E1 and, at high doses, *via* GSH-dependent GSTs. Generally many of the haloalkane datasets support non-genotoxic MOAs for HCC, similar to what is proposed for CTC, with a reactive metabolite inducing cytotoxicity leading to cell proliferation, AHF, and eventually liver tumors. As a demonstration of the consistency and analogy criteria being met, most cancer QSAR and predictive models such as the expert system, OncoLogic have a rule that defined haloalkanes with an increased probability of causing liver cancer; however, this modelling approach is hypothesis generating and does not indicate empirical support for any particular cancer MOA.

Qualitative Rating – Moderate Counter Evidence (-2) (KEs#1 & 2); Strong Counter Evidence (-3) for mutagenic MOA (KE#3); Strong (3) (KEs# 4 & 5).

C. Qualitative and Quantitative Rating of the Key Events for Bradford Hill Causal Considerations

Table 1-4. Qualitative Rating of the Key Events for Bradford Hill Causal Considerations (Step 3).

Bradford Hill Causal Considerations	Key Event #1	Key Event #2	Key Event #3	Key Event #4	Key Event #5
	Metabolism to rxt intermed. & Formation of pro-mutagenic DNA adducts (KE#1)	Insufficient repair or mis-repair of pro-mutagenic DNA adducts (KE #2)	Early induced mutation in cancer critical genes (KE #3)	Cell prolifer'n, clonal expansion of mutant cells, & progression (KE #4)	Hepatocellular carcinoma (HCC) (AO) (KE#5)
Biological Plausibility	Not scored for the case example, but extant data demonstrates not biologically plausible – strong counter (-3)				
Essentiality	No evidence* (0)	No evidence (0)	No evidence (0)	No evidence (0)	No evidence (0)
Empirical Support – Dose and Incidence Concordance	Weak counter evidence (-1)	Moderate counter (-2)	Strong counter (-3)	Moderate (+2)	Strong (+3)
Empirical Support – Temporal Concordance	Weak counter (-1)	Moderate counter (-2)	Strong counter (-3)	Moderate (+2)	Strong (+3)
Consistency & Analogy	Moderate counter (- 2)	Moderate counter (-2)	Strong counter (-3)	Strong (+3)	Strong (+3)

***No evidence of CTC-specific pro-mutagenic DNA adducts**

Considerations & Challenges for CTC MOA quantitative analysis:

CTC presents special challenges in quantitative analysis for a mutagenic MOA because the influential KEs are the early ones related to an early induction of a mutation in critical genes, represented by KE#1, 2, and 3.

The later KEs, 4 & 5, represent converging KEs, ones that are common to other alternative MOAs; they are not informative for a decision on whether a mutagenic MOA is operating or not. This is addressed in part by applying an adjustment factor (10%) to the quantitative weighting of the later KEs (see Table 1-5).

Overall, based on the available data, quantitative scoring for confidence in a mutagenic MOA for CTC induction of liver tumors indicates this MOA is not very probable for CTC. A confidence score of -36 clearly indicates that a mutagenic MOA is not supported by the available data on CTC-induced HCC.

D. Composite Quantification of the WOE for a Mutagenic MOA

Table 1-5. Composite Quantification of the WOE for the Mutagenic MOA (Step 4 and 5).

Bradford Hill Causal Considerations	Key Event #1	Key Event #2	Key Event #3	Key Event #4	Key Event #5
	Metabolism to rxt intermed. & Formation of pro-mutagenic DNA adducts (KE#1)	Insufficient repair or mis-repair of pro- mutagenic DNA adducts (KE #2)	Early induced mutation in cancer critical genes (KE #3)	Cell prolifer'n, clonal expansion of mutant cells, & progression (KE #4) AF: 0.1*	Hepatocellular carcinoma (HCC) (KE#5) AF: 0.1
Essentiality (40%)	0*	0	0	0	0
Empirical Support – (20%) Dose and Incidence Concordance	-0.2	-0.4	-0.6	+0.4	+0.6
Empirical Support – (20%) Temporal Concordance	-0.2	-0.4	-0.6	+0.4	+0.6
Consistency (10%)	-0.2	-0.2	-0.3	+0.2	+0.3
Analogy (10%)	-0.2	-0.2	-0.3	+0.3	+0.3
KE TOTALs Sum = -3.51	-0.8	-1.2	-1.8	+0.13 (1.3 x 0.1)	+0.18 (1.8 x 0.1)

*No evidence of CTC-specific pro-mutagenic DNA adducts.

MOA Confidence Score = -3.51/9.6 (x 100) = - 36.35

*Adjustment Factor of 10% (0.1) applied to late key events due to convergence and lack of specificity to a particular MOA.

E. Key References

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IV. Evaluating the WOE for a Cytotoxic MOA

There is considerable *in vivo* and *in vitro* scientific evidence supporting the mode of action by which carbon tetrachloride produces toxic effects in animals. Mechanistic studies support the following key events in the carcinogenicity of carbon tetrachloride: (KE1) metabolism to trichloromethyl radical by CYP2E1 and subsequent formation of trichloromethyl peroxy radical; (KE2) autocatalytic lipid peroxidation due to the attack on the cellular membrane by the trichloromethyl peroxy radical; (KE3) loss of calcium homeostasis leading to activation of degenerative enzymes and cytotoxicity; (KE4) if exposures above the threshold for hepatotoxicity continue, sustained regenerative and proliferative changes in the liver persist which increases the rate of stem cell division, thereby increasing the probability of fixation of background DNA damage as mutations and an increase in the number of intermediate cell populations that can progress to malignancy; and an increase in cancer incidence (KE#5) (Manibusan et al. 2007, Eastmond, 2008; Borgert et al., 2015).

Provided below is the postulated mode of action in Figure 2-1.

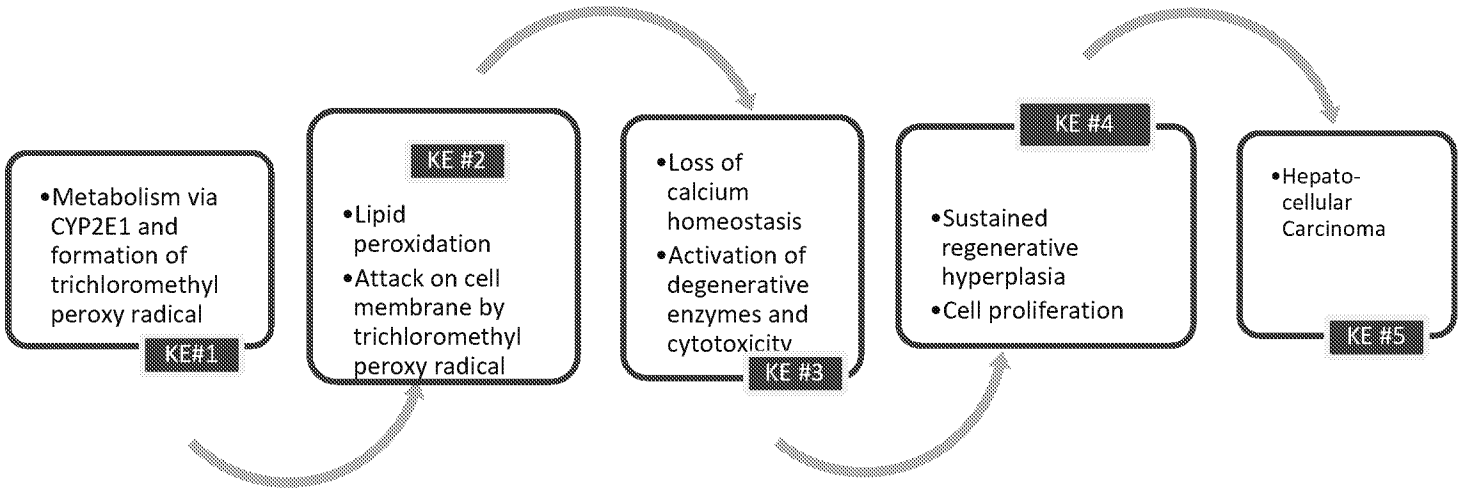

Figure 2-1. Postulated Non-Genotoxic, Cytotoxic Mode of Action for Carbon Tetrachloride.

A. Qualitative Evaluation of the Weight of Evidence (WOE) for Carbon Tetrachloride Acting via a Cytotoxic MOA

Table 2-1. Qualitatively Evaluate the Comparative Weight of Evidence (WOE) for Carbon Tetrachloride Acting via a Cytotoxic MOA (Manibusan et al., 2007; Eastmond, 2008).

Key Event	Supporting Data	Potentially Inconsistent	References
Metabolism via CYP2E1 and formation of Trichloromethyl peroxy radical (KE#1)	Available spin-trapping studies on free-radical products formed by metabolic activation of carbon tetrachloride in rat liver microsomes and *in vivo* in the rat (Albano et al., 1982). Recent studies by Poyer et al. (1980) and Tomasi et al., (1980) have reported successful trapping of CCL3• in liver microsomal fractions with spin trap N-benzylidene-2-methylpropyl-amine N-oxide (PBN). Experiments with PBN give unequivocal proof that CCL3• is produced in isolated liver cells or in liver microsomes exposed to CTC. Studies using CYP450 inhibitors have shown that inhibition of carbon tetrachloride metabolism prevents subsequent liver induced damage. CYP2E1 knockout mice resulted in no liver histopathological effects.	No data available	Poyer et al., 1980 Albano et al., 1982 Tomasi et al., 1980 Martinez et al., 1995; Letteron et al., 1990; Mourelle et al., 1988; Bechtold et al., 1982; Weddle et al., 1976; Wong et al., 1998; Takahashi et al., 2002
Lipid Peroxidation and attack of cellular membranes (KE #2)	Studies of radical scavengers that are not necessarily specific to trichloromethyl peroxy or lipid peroxidative free radicals have shown that these agents confer protection against carbon tetrachloride induced liver toxicity, while another study demonstrated administration of α-tocopherol, Vitamin E antioxidant, had been shown to reduce lipid peroxidation (Gee et al., 1981). Numerous studies have demonstrated lipid peroxidation following carbon tetrachloride exposure by the detection of conjugated dienes in liver lipids, increased exhalation of ethane and pentane (end degradation products of peroxidized polyunsaturated fatty acids) or malondialdehyde and 4-HNE. Hartley et al., (1999) demonstrated the temporal relationship between carbon tetrachloride exposure initiated lipid peroxidation, liver damage and formation of 4-HNE and MDA protein adducts.	No data available	Azri et al., 1991; Gordis et al., 1969; Slater et al., 1981; Packer et al., 1978; Comporti et al., 1985; Gee et al., 1981. Comporti et al., 1984; Lee et al., 1982; Younes et al., 1985; Gee et al., 1981; deZwart et al., 1997; Ichinose et al., 1994; Hartley et al., 1999; Cabre et al., 2000
Loss of calcium homeostasis and initiation of cytotoxicity (KE #3)	Studies have reported 100-fold or more increases in cytosolic concentrations of calcium following exposure to carbon tetrachloride. Studies have demonstrated that effect of carbon tetrachloride on membrane integrity and the active transport that	No data available	Agarwal et al., 1986; Agarwal et al., 1984; Long et al., 1986; Kroner et al., 1982; Lowrey et al., 1981; Moore et al., 1980; Moore et al., 1976;

Key Event	Supporting Data	Potentially Inconsistent	References
	may be by the NADPH-cytochrome P-450 electron-transport chain in liver endoplasmic reticulum, a distance away from the nucleus (Slater, 1972; Recknagel et al., 1977; McCay, 1980), which appear to be secondary to lipid peroxidation.		Hemmings et al., 2002; Racay et al., 1997; Gubskii et al., 1990; Limaye et al., 2003; Slater, 1972; Recknagel et al., 1977 and McCay, 1980
Sustained regenerative cellular proliferation (KE #4)	Administration of 40 mg/kg carbon tetrachloride induced sustained regenerative cellular proliferation, as measured by the increase in BrdU-positive cells in the peri-portal zone at 24 hrs. increasing at 36 hrs. and plateauing at 48 hrs. AHF after 13-wk, only at highest doses	No data available	Glende et al., 1986; Glende et al., 1992; Lee et al., 1998; Eschenbrenner et al., 1946 Nakata et al., 1975; Doolittle et al., 1987 Nagano *et al.*, 2007
Liver tumors (KE #5)	Increased incidence of liver tumors in both rats and mice (see Table 2- 2). Hepatocellular carcinomas appear only at the high dose in rats and mid and high doses in mice, with an all or none response.	No data available	Adervont, 1958; Edwards and Dalton, 1942 Edwards et al., 1942; Della et al., 1961; JBRC et al., 1998

Table 2-2. Incidence of Tumors in F-344 rats and BDF1 mice exposed to carbon tetrachloride vapor for 104 weeks (6hrs/day, 5 days/week). (JBRC et al., 1998)

Tumor	F-344 rats							
	MALE				FEMALE			
	0	5	25	125	0	5	25	125
Adenoma	0/50	1/50	1/50	21/50	0/50	0/50	0/50	40/50
Carcinoma	1/50	0/50	0/50	32/50	0/50	0/50	3/50	15/50
Adenoma or Carcinoma	1/50	1/50	1/50	40.50	0/50	0/50	3/50	44/50
	BDF1 mice							
Adenoma	9/50	9/50	27/50	16/50	2/50	8/49	17/50	5/49
Carcinoma	17/50	12/50	42/50	48/50	2/50	1/49	33/50	48/49
Adenoma or Carcinoma	24/50	20/50	48/50	50/50	4/50	9/49	44/50	48/49

Table 2-3. Dose and Temporal Concordance Table.

Temporal Concordance

Dose Concordance

Temporal	<24hrs.	12-36 hrs.	24hrs	48 hrs. +	2 yrs. Cancer studies
Dose/Conc.	Metabolism to Trichloromethyl Peroxy Radical (KE #1)	Lipid peroxidation and attack on cellular membranes (KE #2)	Loss of calcium homeostasis and initiate cytotoxicity (KE #3)	Sustained regenerative cellular proliferation, hyperplasia (KE #4)	Liver tumors (KE #5) – 2 year cancer studies in mice and rats
800 µL/100 g	+ (rats) based on spin-trapping studies				
5 ppm					
20 ppm		+ (mice)		+ (mice)	
25 ppm					+ (mice)
100 ppm		+ (rats)		++ (mice)	
125 ppm					++ (rats & mice)
270/810 ppm				++ (mice & rats)	
1.59 g/kg	+	+			
50 uM			+		

B. Evolving Bradford Hill Causal Considerations: Qualitative and Quantitative Data Evaluation

Qualitative and quantitative rating categories. [See Becker et al. 2017 for details]

Qualitative	Quantitative	Category description
Strong	3	Multiple studies and/or extensive data provide convincing evidence that the substance causes the KE.
Moderate	2	Some evidence (direct or indirect) indicating the substance causes the KE, but scientific understanding is not yet completely established. There may be some studies that are equivocal.
Weak	1	Very limited evidence (direct or indirect) that the substance causes the KE along this pathway. Scientific understanding of the KE is limited.
No Evidence	0	No data available to support or negate causation of this KE by the substance.
Weak Counter	−1	There is very limited contradictory evidence (direct or indirect) that the substance does not cause this KE.
Moderate Counter	−2	Some evidence (direct or indirect) indicating that the KE is not caused by the substance, but scientific understanding is not completely established. There may be some studies that are equivocal.
Strong Counter	−3	Multiple studies and/or extensive data provide convincing evidence that the substance does not cause this KE.

Essentiality – For key event #1, there is a study by Wong et al., (1998) that demonstrated carbon tetrachloride-induced hepatotoxicity in mice using CYP2E1 knockout mice (cyp2e1 -/-) exposed to 1.59 g/kg carbon tetrachloride via intraperitoneal injection resulted in no increase in AST or serum ALT liver enzyme activity, and an absence of corresponding liver histopathological effects. Conversely, hepatocyte cell lines that over-express cytochrome P450 have increased levels of carbon tetrachloride-induced cytotoxicity. (Jaeschke et al., 2002; Takahashi et al., 2002 and Dai and Cederbaum, 1995).

Qualitative Rating – Strong (3)

Empirical Support (Dose and Incidence Concordance) - Because the earlier key events are demonstrated via *in vitro* assays, the concentrations do not align with the longer term *in vivo* studies. It is clear, however, that the doses for the earlier key events are lower than those needed to elicit liver tumors, which is what you expect to demonstrate for dose concordance that the precursor key events must occur earlier and at lower doses than the tumorigenic dose. The increased incidence of rodent liver tumors is limited to the highest dose tested, a clear threshold type of response (Table 2). Exposure to carbon tetrachloride elicits AHF at 13-wk at high exposures (270 and 810 ppm only), with no tumors, and tumors at doses between 25 ppm in the carcinogenic mouse study and 125 ppm in the chronic/carcinogenicity study in the rat. Data demonstrating dose concordance is moderate to strong, which is based on the good alignment of earlier key events to the tumorigenic dose.

Qualitative Rating – Moderate (2)

Empirical Support (Temporal Concordance) – Cabre et al. (2000) demonstrated temporal concordance between liver lipid peroxidation, glutathione metabolism and development of liver cirrhosis in male Wistar rats exposed to 0.5mL carbon tetrachloride via IP for a total of 9 week, which may be considered a high dose exposure that is relevant to establish empirical support for the cytotoxic key events. By the second week, all livers were fibrotic, and cirrhosis appeared in all treated animals by week 9. Glutathione levels were reduced beginning at week 5 and glutathione peroxidase activity was significantly decreased at week 7 in carbon tetrachloride treated rats. Lipid peroxide-derived aldehydes were elevated starting at week 7. Effects of carbon tetrachloride on cell membrane

integrity and active transport system appear to be secondary (temporally) to lipid peroxidation. Preincubation of liver microsomes with membrane soluble antioxidants protected against the loss of calcium from microsomes and the loss of calcium ATPase activity in a dose- dependent manner. Authors conclude that lipid peroxidation impairs sequestration of calcium ions in microsomes by increasing microsomal membrane permeability and interfering with enzyme activity by altering lipid-calcium pump interactions. Data supporting temporal concordance is considered strong as the data demonstrate lipid peroxidation occurs before loss of calcium occurs and subsequent cell proliferation.

Qualitative Rating – Strong (3)

Consistency and Analogy – Other structurally similar haloalkanes have demonstrated the ability to elicit rodent liver tumors via a nongenotoxic, cytotoxic mode of action; this mode of action typically involves formation of a more reactive metabolite or toxic moiety that causes cell membrane damage, or cytotoxic effects that may lead to sustained regenerative cell hyperplasia and formation of preneoplastic lesions leading to late forming, liver tumors at high doses of exposure. As further demonstration of the consistency and analogy criteria being met, most cancer QSAR and predictive models such as OncoLogic has a rule the defined haloalkanes with an increased probability of causing liver cancer without specifying the mode of action.

Qualitative Rating – Strong (3)

C. Qualitative and Quantitative Rating of the Key Events for Bradford Hill Causal Considerations.

Table 2-4. Qualitative Rating of the Key Events for Bradford Hill Causal Considerations.

Bradford Hill Causal Considerations	Key Event #1	Key Event #2	Key Event #3	Key Event #4	Key Event #5
	Metabolism to Trichloromethyl Peroxy Radical	Lipid Peroxidation and attack on cellular membranes	Loss of calcium homeostasis and initiate cytotoxicity	Sustained regenerative cellular proliferation, hyperplasia	Liver tumors
Essentiality	Strong (+3)	Strong(+3)	Strong(+3)	Strong (+3)	Strong (+3)
Empirical Support – Dose and Incidence Concordance	Moderate (+2)	Moderate (+2)	Moderate (+2)	Moderate (+2)	Strong (+3)
Empirical Support – Temporal Concordance	Strong (+3)	Strong (+3)	Strong (+3)	Strong (+3)	Strong (+3)
Consistency	Strong (+3)	Strong (+3)	Strong (+3)	Strong (+3)	Strong (+3)
Analogy	Strong (+3)	No evidence (0)	No evidence (0)	Strong (+3)	Strong (+3)

D. Composite Quantification of the WOE for a Cytotoxic MOA

Table 2-5 Quantification of the WOE for the Cytotoxic MOA.

Bradford Hill Causal Considerations	Key Event #1	Key Event #2	Key Event #3	Key Event #4	Key Event #5
	Metabolism to Trichloromethyl Peroxy Radical	Lipid Peroxidation and attack on cellular membranes	Loss of calcium homeostasis and initiate cytotoxicity	Sustained regenerative cellular proliferation, Hyperplasia*	Liver tumors*
Essentiality (40%)	1.2	1.2	1.2	1.2	1.2
Empirical Support – (20%) Dose and Incidence Concordance	0.4	0.4	0.4	0.4	0.6
Empirical Support – (20%) Temporal Concordance	0.6	0.6	0.6	0.6	0.6
Consistency (10%)	0.3	0.3	0.3	0.3	0.3
Analogy (10%)	0.3	0	0	0.3	0.3
KE TOTALs Sum = 8.38	2.8	2.5	2.5	0.28 (2.8 x 0.1)	0.3 (3 x 0.1)

Mode of Action Confidence Score = 8.38/9.6 = 87

*Adjustment Factor of 10% (0.1) applied to late key events due to convergence and lack of specificity to a particular MOA.

D. Key References

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V. Conclusions

Two hypothesized MOAs were evaluated for liver tumors induced in rodents by carbon tetrachloride using the quantitative MOA confidence scoring method described by Becker et al., 2017. This method provides a means to compare the WOE for the default MOA (induction of rodent liver tumors *via* a mutagenic MOA) to the WOE for a cytotoxicity and sustained regenerative cellular proliferation MOA.

The confidence scoring results indicate it is highly unlikely that a mutagenic mode of action is plausible for carbon tetrachloride-induced rodent liver tumors. Based on the MOA confidence score of -36, the weight of evidence clearly does not support a mutagenic mode of action for carbon tetrachloride. The negative score indicates there is strong counter evidence for several of the early, diagnostic, KEs for a mutagenic MOA. In other words, the available data indicate that it is highly unlikely that rodent liver tumors are induced by carbon tetrachloride via a mutagenic mode of action.

In contrast, there are significant mechanistic data to support a non-linear, non-genotoxic mode of action for carbon tetrachloride. Based on the MOA confidence score of +87, the weight of evidence clearly supports a cytotoxic mode of action for carbon tetrachloride induction of rodent liver tumors. The MOA causal confidence scoring results indicate that the more likely operative MOA is cytotoxicity and sustained regenerative cellular proliferation. Therefore, an increase in cancer risk would only occur at doses that exceed a specific threshold.

The overall pattern of observations is very consistent with a non-linear, threshold mode of carcinogenic action, as evident by the MOA confidence score of +87 for cytotoxicity compared to the mutagenic MOA score of -36. Therefore, it would be inappropriate to use a linear default for extrapolating cancer risks. Instead, the causal weight of the scientific evidence analysis supports use of a threshold, non-linear method for determining potential cancer risks.

VI. Appendix A. Quantitative Weight of Evidence to Assess Confidence in Potential Modes of Action (Becker et al., 2017)

Quantitative weight of evidence to assess confidence in potential modes of action

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ABSTRACT: The evolved World Health Organization/International Programme on Chemical Safety mode of action (MOA) framework provides a structure for evaluating evidence in pathways of causally linked key events (KE) leading to adverse health effects. Although employed globally, variability in use of the MOA framework has led to different interpretations of the sufficiency of evidence in support of hypothesized MOAs. A proof of concept extension of the MOA framework is proposed for scoring confidence in the supporting data to improve scientific justification for MOA use in characterizing hazards and selecting dose-response extrapolation methods for specific chemicals. This involves selecting hypothesized MOAs, and then, for each MOA, scoring the weight of evidence (WOE) in support of causality for each KE using evolved Bradford Hill causal considerations (biological plausibility, essentiality, dose-response concordance, consistency, and analogy). This early proof of concept method is demonstrated by comparing two potential MOAs (mutagenicity and peroxisome proliferator activated receptor-alpha) for clofibrate, a rodent liver carcinogen. Quantitative confidence scoring of hypothesized MOAs is shown to be useful in characterizing the likely operative MOA. To guide method refinement and future confidence scoring for a spectrum of MOAs, areas warranting further focus and lessons learned, including the need to incorporate a narrative discussion of the weights used in the evaluation and an overall evaluation of the plausibility of the outcome, are presented.